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Prediction of the three-dimensional (3D) Structure of Somatostatin Receptor Type 1 of *Homo sapiens* by Homology Modelling

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ABSTRACT

Somatostatin (growth hormone-inhibiting hormone (GHIH) or somatotropin release-inhibiting factor (SRIF) is recognized as an important mediator and plays great role between the nervous and the immune system along with cancer. Somatostatin (SST) is a hypothalamic, peptide and pancreatic hormone that regulates the endocrine system and cell proliferation via G protein-coupled receptors family. Somatostatin, acting through the 5 somatostatin receptors although the functional role of its receptors (sst(1)-sst(5)) is poorly understood in humans. Despite it consists of thousands of integral membrane proteins exert a wide variety of physiological functions and response to a large portion of the drug targets, but the three dimensional (3D) structure of Somatostatin receptor type 1 [*Homo sapiens*] has not yet been reported in Protein Data Bank. This is the first report on predicting a three dimensional (3D) structure for Somatostatin receptor type 1 of *Homo sapiens*. The three dimensional (3D) structure for Somatostatin receptor type 1 was generated through Homology modeling by Swiss model work space Server, the quality of generated model was performed by SAVES server and Rampage program. The present protein structure prediction and validation of generated protein structure may provide promising opportunities for drug discovery as drug target, secreting pituitary tumors research for cancer prevention and treatment and furnishes an adequate foundation for functional analysis of experimentally derived crystal structures.

Keywords: Somatostatin receptor, 3D Structure prediction, Homology modeling, SAVES Server,

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INTRODUCTION

Somatostatin (SST) is ubiquitously expressed in humans, with high concentrations in brain, liver, lungs, pancreas, thyroid, gastrointestinal tract, and adrenal gland mainly acting as an inhibitor of exocrine and endocrine secretions on target organs [1]. SST is a tetradecapeptide that is widely distributed in the body and it is a peptide hormone that regulates diverse cellular functions such as neurotransmission [2], cell proliferation [3, 4], and endocrine signaling [5, 6] as well as inhibiting the release of many hormones and other secretary proteins [7-9] and different aspects form the basis for the therapeutic potential of somatostatin in various diseases [10].Somatostatin (SST) is a small cyclic neuropeptide containing a disulfide bond linking the cysteine residues at positions 3 and 14 (Cys3-Cys14). Native SST has two molecular forms, SST-28 and SST-14, consisting of 28 or 14 amino acid [12-13].

SST receptors are differentially expressed in discrete distribution in multiple target organs, such as central nervous and immune systems, pituitary, thyroid, and adrenal glands, pancreas, gut, kidney and also localized in the peripheral nervous system and play multiple subtypes in a tissue-specific pattern and distinct physiological roles [14]. SST receptors are widely expressed throughout the brain: in particular, sst1 and sst2 have a diffuse localization, whereas sst4 and sst5 show a more confined expression in hippocampus and hypothalamus, recent evidence proposed a key role of SST1 neuron dysfunction in Alzheimer's disease [7] and other brain disorders, involved in the arrest of cell cycle progression [15, 16]. SST1 is a major source of gut influencing motility, secretion and absorption [17]. SST1 receptor expression was observed in peritumoral vessels, mainly in endothelial cells, brain tumor, prostate cancer, breast cancer [18-20]. Gastroenteropancreatic neuroendocrine tumours (GEP NETs) are a heterogeneous group of relatively rare tumours and around 80% of GEP NETs express somatostatin receptors (SSTRs), located on the cell membrane. There are five different G-protein coupled receptor subtypes (SSTRs 1-5) that are differently expressed in the various types of tumour [21-23]. Homo or hetero demonization of all GPCR family, including SST receptors, plays a fundamental role in ligand binding, receptor expression, trafficking and desensitization, and signal transduction because biological effects of somatostatin are probably mediated by a family of G protein-coupled receptors that are expressed in a tissue-specific manner. At pituitary gland level, sst1 and sst5 mainly control GH and prolactin secretion while sst2 is involved in the release of GH, TSH, and ACTH [3].

SST controls cancer cell proliferation via the interference with different signaling pathways (PTPs, JAK2, Ras/ERK, and Pi3K/Akt) resulting in cytostatic effects mediated by the induction of involving the induction of cell cycle inhibitors p27 or p21, or tumor suppressors, like Zac1[24] and GEP-NETs display high expression of SST1 receptors. The biological effects induced after SST1 receptor activation allowed their identification as relevant drug targets by different pathways. Thus SST1 analogs are potentially useful for the diagnosis and therapy of these tumors, cancer, neurotransmission and endocrine signaling.SSR1 are ability to protect from above diseases because of peptide hormone is a better drug target especially for obese and cancer [25-28].

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In the present study homology based 3-dimensional structure prediction of Somatostatin receptor type 1 [*Homo sapiens*] and their characterization was carried out. The secondary structure tells about the possible alpha helices, beta sheets and coiled regions in the Somatostatin receptor type 1. The Ramachandran plot tells about the residues which have favorable conformation for psi and phi angles which will further help in understanding the stability and rotation of amino acid in protein structure and that is play an important bridging role in comparative modeling of this protein (structural evidence) may be helpful for a new direction for disease control with positive pharmaceutical efficiency.

MATERIALS AND METHODS

Retrieval of the target sequence

The amino acid sequence of Somatostatin receptor type 1 [*Homo sapiens*] was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) (Acc. No: NP_001040).

Sequence analysis and protein disorder Prediction

After retrieval of this sequence ProtParam [28] was used to predict the physiochemical properties. ProtParam computed the molecular weight, theoretical pI, amino acid composition, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). Protein disorder was predicted using DisEMBL [29]. A great challenge in the proteomics and structural genomics era is to predict protein structure and function, including identification of those proteins that are partially or wholly unstructured. DisEMBL is a computational tool for prediction of disordered/unstructured regions within a protein sequence.

Template Selection

The protein sequence of Somatostatin receptor type 1 was retrieved from NCBI (Acc. No: Acc. No: NP_001040). A BLASTP [30] search with default parameter was performed against the Brook Heaven Protein Data Bank (PDB) [31] to find suitable template for homology modeling. A set of PDB structures i.e. 2EA3, 4DJH, 4EJ4, and 4DKL were showing close similarity with the target sequence. Basing on maximum identity with high positives and lower gap percentage (%) (Shown in Table 1), Crystal Structure of the Delta Opioid Receptor Bound to Naltrindole (PDB ID: 4EJ4_A) was selected as a template. The percentage of sequence identity and positives was 48% and 67% respectively between the template and the target.

Multiple Sequence Alignment (MSA) is used to align more than two sequences thereby representing the occurrence of one or more patterns common to a set of sequences, regions of variations and the nature of the mutations/substitutions. COBALT [32] is an online tool used for the construction of MSA. The hits obtained by performing BLASTP were aligned with query using.

COBALT, a constraint based alignment tool that implements a general framework for multiple alignments of protein sequences. COBALT finds a collection of pair wise constraints derived from database searches, sequence similarity and user input, combines these pair wise constraints, and then incorporates them into a progressive multiple alignment. We show that using constraints derived from the conserved domain database (CDD) and PROSITE protein-motif database improves COBALT's alignment quality. We also show that COBALT has reasonable runtime performance and alignment accuracy comparable to or exceeding that of other tools for a broad range of problems. An optimal alignment between the target sequence (Somatostatin receptor type 1) and templates (PDB ID: 2EA3_A, 4DJH_A, 4EJ4_A and 4DKL_A) was obtained by COBALT and Clustal W used to alignment between template and target preotein sequence. Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2/)ClustalW is a widely used system for aligning any number of homologous nucleotide or protein sequences. This heuristic approach is necessary because finding the global optimal solution between a two or more sequence and the algorithm starts by computing a rough distance matrix between each pair of sequences based on pair wise sequence alignment scores [33]

Secondary structure prediction

SOPMA [34] was employed for computing and analyzing the secondary structural features of Somatostatin receptor type 1 protein sequence.

Three-dimensional (3D) structure prediction using homology approach

3D structure of protein was determined by homology modeling. BLASTP search with default parameters against the Protein data bank (PDB) was used to find the best suitable templates for homology modelling. The homology modeling structure prediction was done using 4EJ4A as template model Structure of Somatostatin receptor type 1 by SWISS-MODEL Server. SWISS-MODEL (http://swissmodel.expasy.org/) [35-37] is a fully automated web-based environment for protein structure homology modelling server.

Model Validation

The 3-dimensional structure prediction was carried out by alignment of target sequences with template structures (retrieved from PDB database), using automated web-based environment for protein structure homology modelling server (SWISS-MODEL server). Further, the model was assessed by VARIFY 3D [38]. Structural validation of protein model was done by Rampage [39] which determines stereo chemical aspects along with main chain and side chain parameters with comprehensive analysis .The Ramachandran plot of Somatostatin receptor type 1 protein, shows that various residues falling under allowed, favoured and in disallowed regions.

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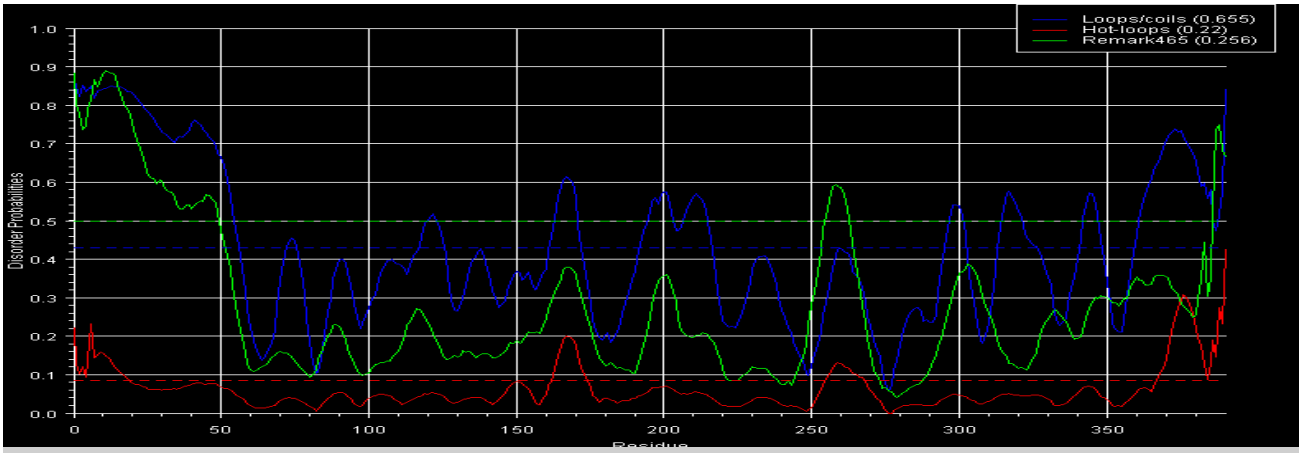
RESULTS AND DISCUSSION

After retrieval of Somatostatin receptor type 1 protein sequence ProtParam was used to predict the physiochemical properties. The protein sequence was predicted to be 391 amino acids, with a molecular weight of 42686.1 Daltons, an isoelectric point of 8.68, an isoelectric point above 7 indicates a positive charged protein, an instability index of 52.30 and Aliphatic index is 105.17.The positive GRAVY index of 0.488 is indicative of a hydrophobic and insoluble protein.

Protein disorder or disordered/unstructured regions within a protein sequence was predicted using DisEMBL server and the results shows below (Fig. 1)



Disordered by Loops/coils definition

```
>Somatostatin receptor type 1_LOOPS 1-57, 118-126, 163-172, 193-217, 315-327, 342-349, 361-391
MFPNGTASSP SSSPSPSPGS CGEGGGSRGP GAGAADGMEE PGRNASQNGT LSEGQGSail isfiysvvcl vglcgnsmvi yvilryakmk tatniyilnl aiadellmls
vpflvtsTLL RHWPFGallc rlvlsvdavn mftsiycltv lsvdryvavv hpIKAARYRR PTvakvvnlg vwvlslllvil piVVFSRTAA NSDGTVACNM LMPEPAQrwl
vgfvlytflm gfllpvgaic lcyvliiakm rmvalkagwq qrkrserkit lmvmmvvmvf vicwmpfyvv qlvnvfaeqd datvsqlsvi lgyaNSCANP ILYGFLSdnf
krsfqrilcl sWMDNAAEEp vdyyatalks RAYSVEDFQP ENLESGGVFR NGTCTSRITT L
```

Disordered by Hot-loops definition

```
>Somatostatin receptor type 1_HOTLOOPS 1-19, 164-174, 256-268, 369-391
MFPNGTASSP SSSPSPSPGs cgegggsrgp gagaadgmee pgrnasqngt lsegqgsail isfiysvvcl vglcgnsmvi yvilryakmk tatniyilnl aiadellmls
vpflvtstll rhwpfgallc rlvlsvdavn mftsiycltv lsvdryvavv hpiKAARYRR PTVAkvvnlg vwvlslllvil pivvfsrtaa nsdgtvacnm lmpepaqrwl
vgfvlytflm gfllpvgaic lcyvliiakm rmvalKAGWQ QRKRSERKit lmvmmvvmvf vicwmpfyvv qlvnvfaeqd datvsqlsvi lgyanscanp ilygflsdnf
krsfqrilcl swmdnaaeep vdyyatalks raysvedfQP ENLESGGVFR NGTCTSRITT L
```

Disordered by Remark-465 definition

```
>Somatostatin receptor type 1_REM465 1-50
MFPNGTASSP SSSPSPSPGS CGEGGGSRGP GAGAADGMEE PGRNASQNGT lsegqgsail isfiysvvcl vglcgnsmvi yvilryakmk tatniyilnl aiadellmls
vpflvtstll rhwpfgallc rlvlsvdavn mftsiycltv lsvdryvavv hpikaaryrr ptvakvvnlg vwvlslllvil pivvfsrtaa nsdgtvacnm lmpepaqrwl
vgfvlytflm gfllpvgaic lcyvliiakm rmvalkagwq qrkrserkit lmvmmvvmvf vicwmpfyvv qlvnvfaeqd datvsqlsvi lgyanscanp ilygflsdnf
krsfqrilcl swmdnaaeep vdyyatalks raysvedfqp enlesggvfr ngtctsritt l
```

(Figure. 1 Protein sequence disorder prediction by DisEMBL)

The template (NOFQ OPIOID RECEPTOR) and target (Somatostatin receptor type 1) sequence alignment result was predicted by ClustaW and the result page shows below (Fig 2).

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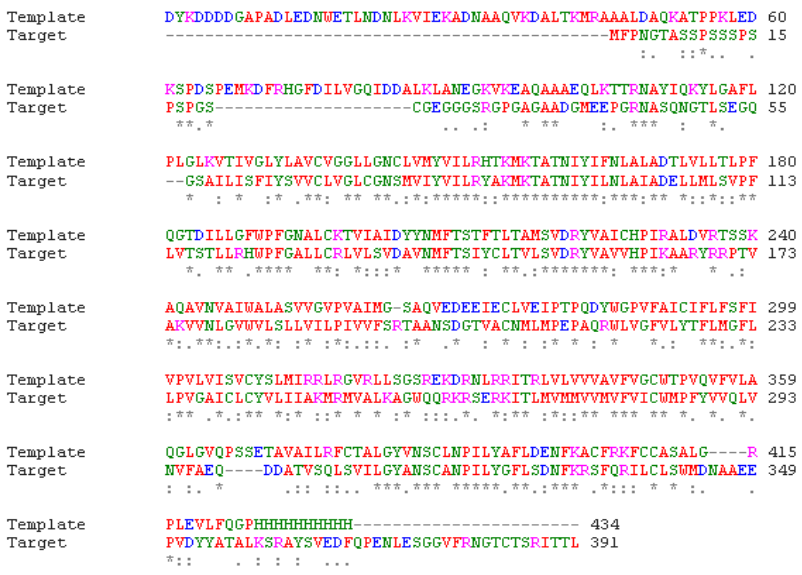
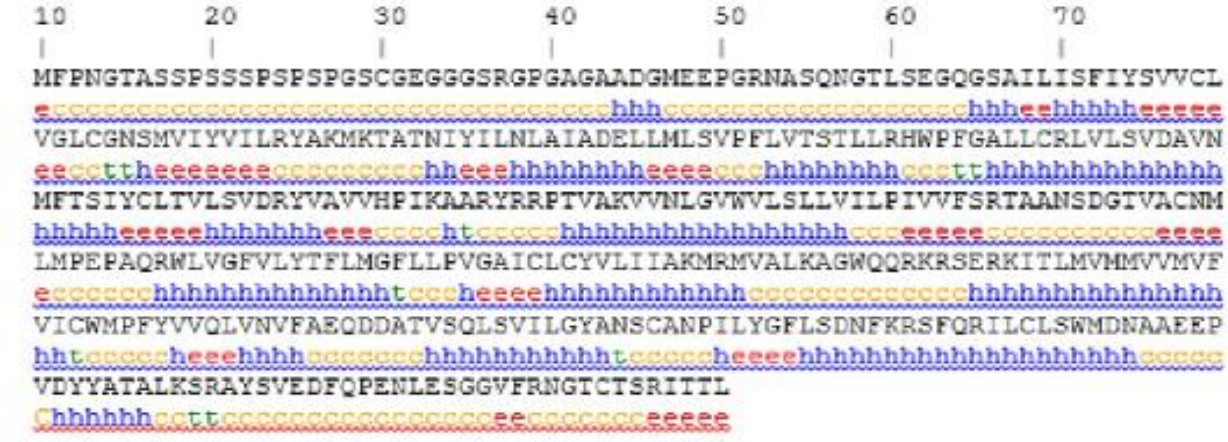


(Figure 2 Sequence Alignment by using ClustalW)

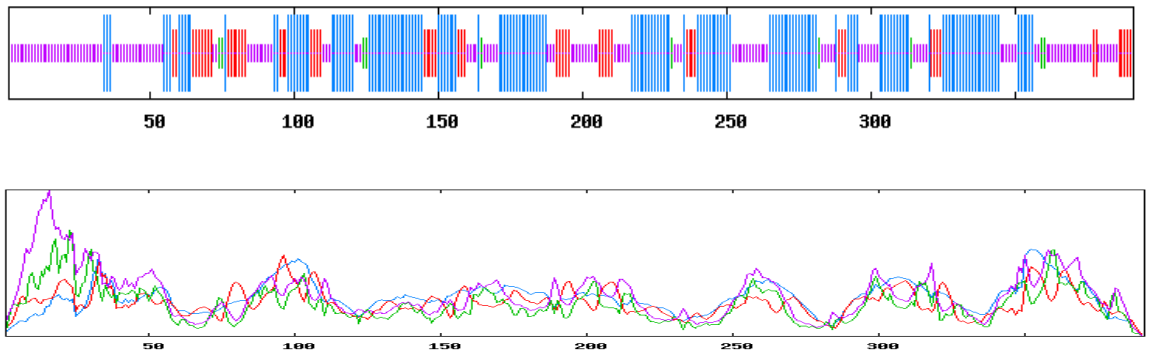
In this study the protein sequence of Somatostatin receptor type 1 [*Homo sapiens*] was retrieve from NCBI database and sequence was checked for their suitability for homology modelling using BLASTP analysis (Table 1).

PDB ID	Max. Identity	Positives	Gap
2EA3	44%	62%	2%
4DJH	42%	63%	5%
4EJ4	48%	67%	1%
4DKL	47%	66%	1%

Secondary structure analysis was performed using SOPMA **(**Fig.3) and the protein was predicted to contain several helices, consistent with the ProtParam results.

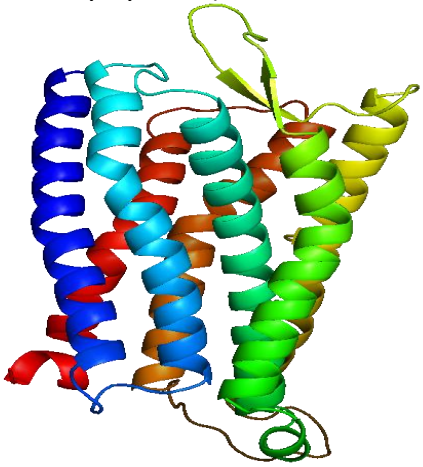


ISSN: 0975-8585



(Figure 3 Secondary structure prediction using SOPMA)

The 3D model that was obtained using the suitable templates (4EJ4_A) by the above mentioned approach was visualized by PyMOL (academic version) software package (Fig. 4).



(Figure 4. The 3D protein structure of Somatostatin receptor type 1 by PyMOL) software)

The stereo chemical quality of the model was assessed using RAMACHANDRAN PLOT analysis (Fig. 5).

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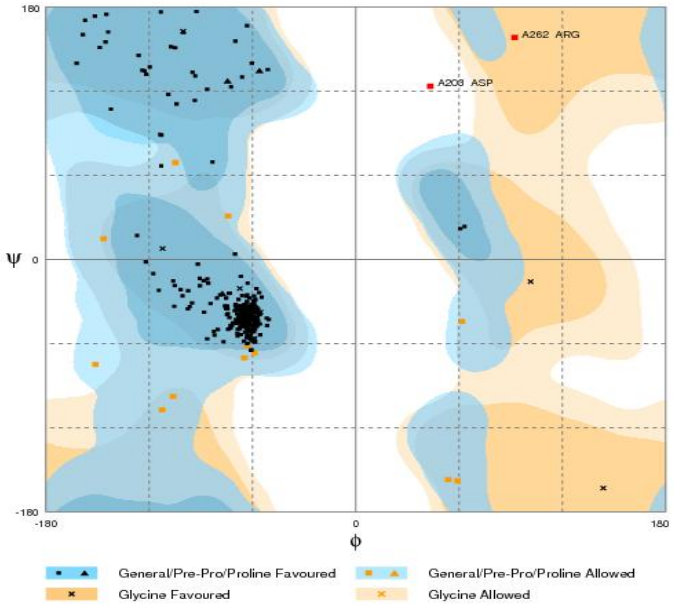

(Figure. 5 Rampage result page)

Number of residues in favoured region : 95.0%
Number of residues in allowed region : 4.3%
Number of residues in outlier region : 0.7%

Further studies using Verify 3-D were also carried out to check the reliability of the model. The plot indicates that maximum number of amino acid (95%) residues in favoured region and only 0.7% of the residues lie in the disallowed region. The generated model thus obtained in this study is the best model of Somatostatin receptor type 1 [*Homo sapiens*].

CONCLUSION

The main objective of this study was to perform sequence analysis, structure analysis and homology modelling of Somatostatin receptor type 1 [*Homo sapiens*]. The *in-silico* approach helps the researchers by giving them an in-hand idea so that they can happily advance towards the treatment of the disease and helps to minimize the gap between *in silico* and wet lab for determination of 3D structure of a protein. Also work aims to prove that no disease is incurable but the cure may be hidden in some other form. Also this work aims to highlight application of Bioinformatics in Drug Designing. The 3D structure model of Somatostatin receptor type 1 of *Homo sapiens* was stable and proved reliable using the Rampage and VERIFY3D module. The maximum amino acids fall under α-helix region which provides stability to the protein and overall results provided the evidences that the predicted 3D structure of Somatostatin receptor type 1 is acceptable and of good quality which can be further extended with the identification of receptor–ligand study for cancer treatment.

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