J Endocrinol Reprod 16 (2012) 2 : 57-64 ***Original Article***

In silico structural analysis and characterization of HUMAN KiSS-1 receptor: A metastasis suppressor protein in melanomas and breast cancer

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Summary

Metastasis, a major cause of death in cancer patients, involves the spread of a tumor or cancer to distant parts of the body as primary cancer, invasion of surrounding tissue, spread through circulation, re-invasion and proliferation in distant organs. KiSS1 is a metastasis-suppressor protein that suppresses metastases in malignant melanomas and in some breast carcinomas, without affecting tumorigenicity and also may be mediated in part by cell cycle arrest and induction of apoptosis in malignant cells. To understand the operational mechanism, structural model is always important. Therefore, in present study a complete structural analysis and three- dimensional (3D) modeling of KiSS-1 receptor, with a molecular weight of 42,586 kDa, of *Homo sapiens* was carried out. The 398 amino acid sequence of the KiSS-1 receptor protein was retrieved from Uniprot KB database (Acc. no: **Q969F8)**. Based on the PDB Blast result and analysis the three-dimensional structure of **KiSS-1R** was predicted by using the SWISS MODEL, ESyPred 3D protein comparative modeling server. The predicted model was further assessed by Rampage, VERIFY-3D and PROCHECK graph with acceptable scores. The overall result provides evidence of good quality of model and furnishes an adequate foundation for functional analysis of experimentally derived crystal structures and also helps in understanding metastasis.

Key words : **KiSS-1R**, BLASTp, SWISS MODEL, UniProtKB, SAVES server

Introduction

Metastasis genes are involved in a complicated series of events that includes the separation of single cells from a solid tumor, venous invasion, immunologic escape in the circulation, adhesion to endothelial cells, extravasation from lymph-and blood vessels, proliferation and induction of angiogenesis (Nakayama et al., 2012). Right now, approximately 30 canonical metastasis suppressor genes have been identified in various cancers (Cook et al., 2011; Thiolloy et al., 2011). KiSS1 and KiSS1R are among the putative metasis suppressor genes in melanoma and breast cancer, encoding kisspeptins (Marot et al., 2007; Lee et al., 1996, 1997).

KiSS1 was originally identified as a metastasis-suppressor gene capable of inhibiting tumor progression and may be involved in biology of pituitary tumors (Martinez-Fuentes et al., 2011; Hata et al., 2007). Kisspeptin is a G-protein coupled receptor ligand for GPR54 (Messager et al., 2005; Muir et al. 2001, Kotani et al., 2001; Cho et al., 2012). In recent research it has became clear that kisspeptin-GPR54 signaling plays an important role in initiating secretion of gonadotropin-relasing hormone (GnRH) at puberty, the extent of which is an area of ongoing research (Smith et al., 2006; Kotani et al., 2001; Cho, 2010).

KiSS-1 expression is increased in human breast cancer, particularly in patients with aggressive tumors and with mortality. Over-expression of KiSS-1 in breast cancer cells results in a more aggressive phenotype. Together, it suggests that KiSS-1 plays a role beyond the initial metastasis repression in this cancer type (Martin et al., 2005).

Developing a 3-Dimensional structure with reference to the sequence helps in further modification of the structure and, hence, it might play a vital role in increasing the efficiency of the breast cancer research. The structure can be developed computationally, which can be predicted and validated through Homology Modeling. Approaches can be made for identification of various active sites for the binding of receptors through servers and tools, which may lead in identification of most portable site for the protein.

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Materials and Methods

Target selection

The amino acid sequence of KiSS-1 receptor of *Homo sapiens* was retrieved from the *UniProtKB* (Acc. No.: Q969F8) database (http://www.uniprot.org /help/ uniprotkb).

Template selection

A $BLAST_P$ (Altschul et al., 1990) search with default parameters was performed against the Brook Heaven Protein Data Bank (PDB) (Berman et al., 2000) to find suitable template for homology modeling. A set of PDB structures i.e. 4EA3_A, 4DJH_A, 4DKL_A, 3KJ6_A, 4R4R_A were showing close similarity with the target sequence. Basing on maximum identity with high positives and lower gap percentage (%) (Table 1), structure of the NOFQ OPIOID RECEPTOR IN COMPLEX WITH A PEPTIDE Mimetic (4EA3_A) was selected as the template since the percentage of Query coverage, Max. Identity and Gap between the template and the target was 74%, 37% and 7%, respectively.

Construction of homology model

SWISS MODEL Workshop, ESyPred 3D server is used for homology or comparative modeling of protein three-dimensional structures. The user provides an alignment of a sequence to be modeled with known related structures and automatically calculates a model containing all non-hydrogen atoms. Server implements comparative protein structure modeling by satisfaction of spatial restraints, and can perform many additional tasks, including *de novo* modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. (Sateesh et al. 2010; Schwede et al. 2003, Arnold , 2006; Kiefer et al. 2009; Lambert et al., 2002).

The secondary structural features of protein that was employed in SOPMA view (Combet et al., 2000), a new highly accurate secondary structure prediction method, was adopted in this study. SOPMA incorporates two feed-forward neural networks which perform an analysis on output obtained from PSI-BLAST (position specific Iterated BLAST) (Altschul et al., 1997).

The 3-dimensional structure prediction model was assessed by VERIFY 3D (http://nihserver.mbi.ucla.edu/ Verify_3D/) visualization protein model (Eisenberg et al., 1997). This was carried out adopting PyMol software (DeLano, 2002). Structural validation of protein model was done by Rampage (http://mordred.bioc.cam.ac.uk/ ~rapper/rampage.php) which determines stereo-chemical aspects along with main chain and side chain parameters with comprehensive analysis (Lovell et al., 2003). The Ramachandran plot of KiSS1R protein shows that various residues are falling under allowed, favored and regions.

Results and Discussion

In this study the KiSS1R has been retrieve from UniprotKB database and sequence was checked for suitability for homology modeling using BLASTp analysis.

Table 1: BLASTp report of KiSS1R

PDB ID	Query coverage	E. value	Gap	Max. Identity
4EA3_A	74%	3e-40	7%	37%
4DJH_A	70%	5e-25	7%	40%
4DKL_A	80%	4e-24	10%	36%
3KJ6_A	85%	5e-22	7%	25%
4R4R_A	85%	6e-22	10%	25%

The alignment score of target and template are shown below:

```
TARGET    1            WLV PLFFAALMLL GLVGNSLVIY VICRHKPMRT VTNFYIANLA
4ea3A     47    plglk--vti vglylavcvg gllgnclvmy vilrhtkmkt atniyifnla

TARGET                  hh hhhhhhhhhh hhhhhhhhhh hhhh       hhhhhhhhhh
4ea3A              hhhh  hhh hhhhhhhhhh hhhhhhhhhh hhhh       hhhhhhhhhh


TARGET    44    ATDVTFLLCC VPFTALLYPL PGWVLGDFMC KFVNYIQQVS VQATCATLTA
4ea3A     95    ladtlvl-lt lpfqgtdill gfwpfgnalc ktviaidyyn mftstftlta

TARGET          hhhhhhh    hhhhhhhhhh         hhh hhhhhhhhhh hhhhhhhhhh
4ea3A           hhhhhhh hh hhhhhhhhhh         hhh hhhhhhhhhh hhhhhhhhhh


TARGET    94    MSVDRWYVTV FPLRALHRRT PRLALAVSLS IWVGSAAVSA PVLALHRLSP
4ea3A     144   msvdryvaic hptsska--- ----qavnva iwalasvvgv pvaimgsaqv

TARGET          hhhhhhhh    hhh   hhh hh    hhhh hhhhhhhhhh hhhhh ssss
4ea3A           hhhhhhhhhh     hhh        hhhhhh hhhhhhhhhh hhhhh sss


TARGET    144   GP-RAYCSEA FP--SRALER AFALYNLLAL YLLPLLATCA CYAAMLRHLG
4ea3A     194   edeeieclve iptpqdywgp vfaiciflfs fivpvlvisv cyslmirrlr

TARGET          s  sssss          hhh hhhhhhhhh    hhhhhhhh hhhhhhhhh
4ea3A                sss       hhhhhh hhhhhhhhh    hhhhhhhh hhhhhhhhh


TARGET    191   RVAVRPAPAD SALQGQVLAE RAGAVRAKVS RLVAAVVLLF AACWGPIQLF
4ea3A     244   gvrllsg--- --------sr ekdrnlrrit rlvlvvvavf vgcwtpvqvf

TARGET                 sss s   ssss      hhhhhhh hhhhhhhhhh hh   hhhhh
4ea3A                                  hhhhhhhhhh hhhhhhhhhh hh   hhhhh


TARGET    241   LVLQALGPAG SWHPRSYAAY ALKTWAHCMS YSNSALNPLL YAFLGSHFRQ
4ea3A     283   vlaqglgvqp s----setav ailrfctalg yvnsclnpil yafldenfka

TARGET          hhhhh            hhhh hhhhhhhhhh hhhhhhhhhh hh   hhhhh
4ea3A           hhhhhh           hhhh hhhhhhhhhh hhhhhhhhhh hh   hhhhh


TARGET    291   AFR
4ea3A     329   cfr-

TARGET
4ea3A           h
```

Secondary and tertiary structure prediction

The secondary structures of proteins are the regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha-helix and beta-sheet. SOPMA view showed to be 175 helices (43.97%), 57 strands (14.32%), 155 coils (38.94%) and 11 (2.7%) beta turn present at various positions in the KiSS1R protein structure of *Homo sapiens* (Fig.1).

```
        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MHTVATSGPNASWGAPANASGCPGCGANASDGPVPSPRAVDAWLVPLFFAALMLLGLVGNSLVIYVICRH
heeeeccccceeeccccccccccccccccccccccccccchhecchhheeeeeeeccttheeeeeeecc
KPMRTVTNFYIANLAATDVTFLLCCVPFTALLYPLPGWVLGDFMCKFVNYIQQVSVQATCATLTAMSVDR
ccchhhheeeehhhhhhheeeeeccccchheeeeccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
WYVTVFPLRALHRRTPRLALAVSLSIWVGSAAVSAPVLALHRLSPGPRAYCSEAFPSRALERAFALYNLL
hheeecchhhccccccheeeehhhhhhhhhhhcccheeeeccccccceeeeeccccchhhhhhhhhhhh
ALYLLPLLATCACYAAMLRHLGRVAVRPAPADSALQGQVLAERAGAVRAKVSRLVAAVVLLFAACWGPIQ
hhhhcchhhhhhhhhhhhhhhhhcccccccccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhtcccch
LFLVLQALGPAGSWHPRSYAAYALKTWAHCMSYSNSALNPLLYAFLGSHFRQAFRRVCPCAPRRPRRPRR
hhhhhhhccccccccccchhhhhhhhhhhhhhhctttcccheeehhtccchhhhhhhccccccccccccc
PGPSDPAAPHAELLRLGSHPAPARAQKPGSSGLAARGLCVLGEDNAPL
ccccccccchhhhhhhccccccccccttcccccccccceeehhttccc
```

Sequence length: 398

Protein Structural Unit	No. of amino acids	Percentage of Structural Unit
Alpha helix (Hh)	175	43.97
3 ten helix (Gg)	0	0
Pi helix(Ii)	0	0
Beta bridge(Bb)	0	0
Extended strand(Ee)	57	14.32
Beta turn (Tt)	11	2.7
Bend region (Ss)	0	0
Random Coil (Cc)	155	38.94
Ambigous states	0	0
Other states	0	0

Sequence length: 398

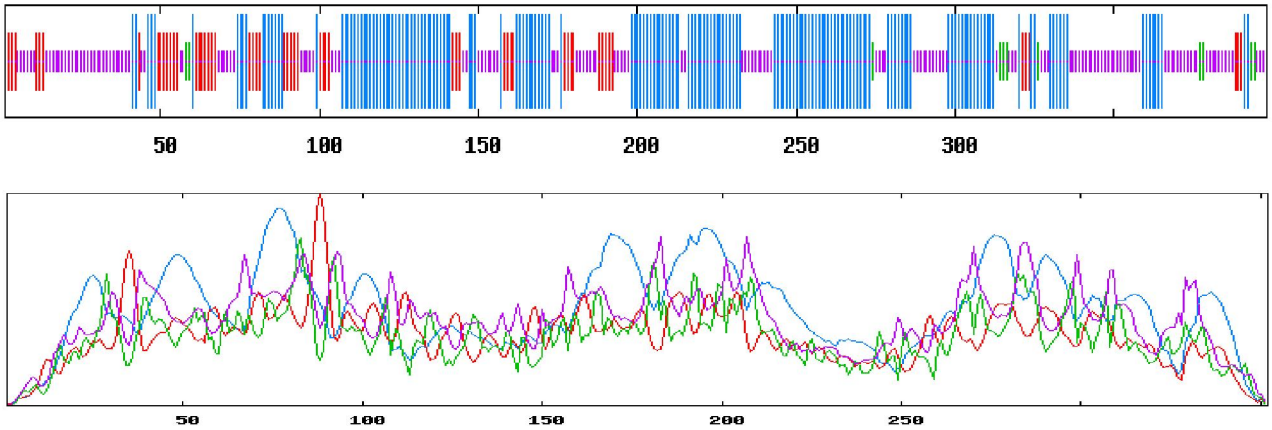

Fig.1 Secondary Structure prediction result of KiSS1R protein

Tertiary structure

After choosing a suitable template (4EA3_A), the model was constructed for the target protein using SWISS MODEL and ESyPred 3D (comparative Protein 3D modeling server). The predicted model was visualized under PyMol visualization software. 3-D structure of KiSS1R is given below (Fig .2).

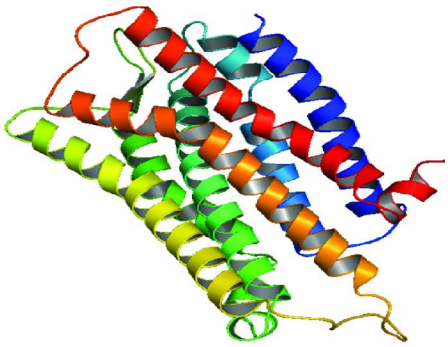

Fig. 2 Three- Dimensional Structure of KiSS1R

Protein model validity

The geometrical and structural consistencies of both modeled and template proteins were evaluated by different approaches. The structural validation was carried out by PROCHECK, a well known protein structure checking program which expounds the Φ and Ψ distributions of Ramachandran plot. This analysis revealed that only three residues (1.2%) in Ramachandran plot of KiSS1R protein fall under disallowed region. Overall, both homologies have nearly same distribution in the steriochemically allowed main chain atoms (91.5%) (Fig. 3).

In addition, two more protein evaluation programs (Verify3D and ERRAT) were utilized to check the stereochemistry of our model. VERIFY 3D (Fig. 4) scores the compatibility between the amino acid sequence and the environment of the amino acid side chains in the model. It assesses the environment of the side chain based on the solvent accessibility and the fraction of side chain covered by polar atoms. ERRAT assesses the arrangement of different types of atoms with respect to one another in the protein model. It is a sensitive technique, which is good for identifying incorrectly folded regions in preliminary protein models.

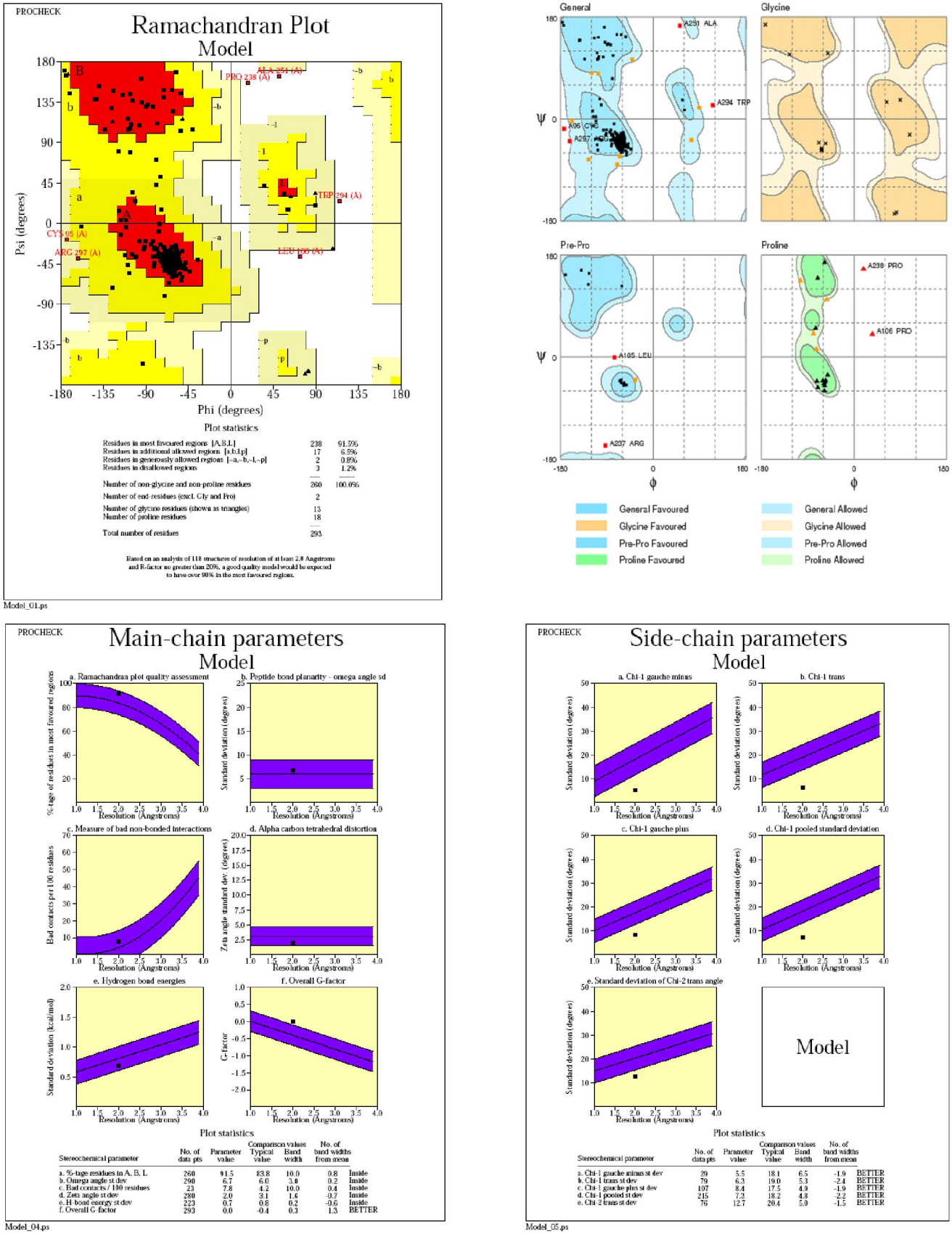

Fig. 3 Protein validation study by SAVE and Rampage Server

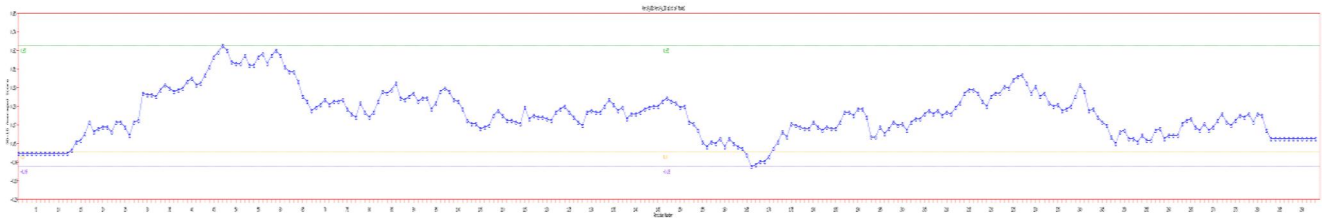

Fig.4. VERIFY 3D graph of KiSS1R protein

ERRAT plot (Fig. 5) shows that the developed structure of KiSS1R is acceptable. (Overall quality factor 81.053%).

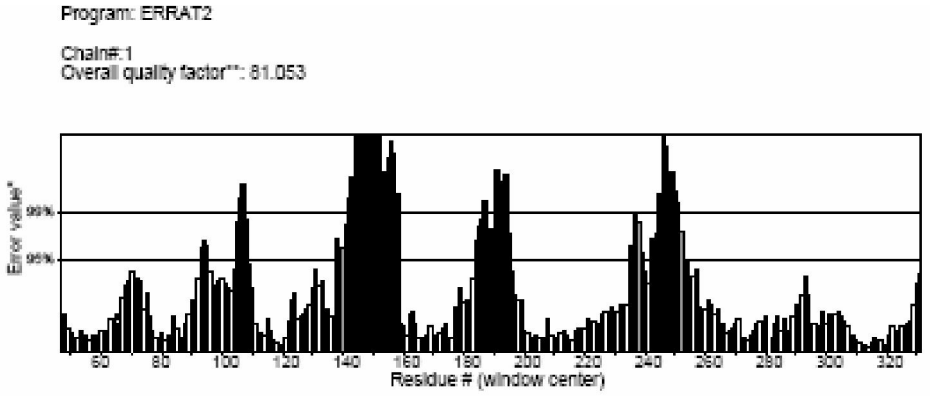

Fig. 5 ERRAT plot of KiSS1R protein

Conclusion

The purpose of this study is to minimize the gap between *in silico* and wet lab determination of 3D structure of a protein by molecular modeling. The 3D structure model of KiSS1R protein was stable and proved reliable using the PROCHECK and VERIFY3D module. The maximum amino acids fall under α-helix region which provide stability to the protein. The overall results provide evidence that the predicted 3D structure of KiSS1R protein is acceptable and of good quality, and predicting the structure for KiSS1R will give an idea of its active site and the active site residues which can be further analyzed by the use of software's for preparing ligands and receptors along with cancer research.

Acknowledgments

We gratefully acknowledge the encouragement and support of Dr. Tirupati Panigrahi, Chairman, Hi-Tech Group of Institutions. We are thankful to faculty members of BJB (A) College, Bhubaneswar, Konark Institute of Science and Technology, Bhubaneswar, for their help and encouragement in preparing the manuscript.

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