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Binding Interaction Analysis of Neuromedin U Receptor 1 with the Native Protein Neuromedin U

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Abstract

Neuromedin, a neuropeptide, which is involved in various functions that include contractile activity on smooth muscle, controlling the blood flow and ion transport in the intestine, increased blood pressure and regulation of adrenocortical function. It is involved in the pathophysiology of various immune mediated inflammatory diseases like asthma. In this study, we have performed protein-protein docking analysis of neuromedin U – neuromedin U receptor 1 complex. We have developed homology models of neuromedin U, and selected a reliable model using model validation. The model was docked with the receptor model, to analyse the crucial interactions of the complex. This study could be helpful as a tool in developing novel and potent drugs for the diseases related with neuromedin U receptor 1.

Keywords: Neuromedin U Receptor 1, GPCR, Neuromedin, NMUR1, Protein-protein Docking

1. Introduction

Neuromedin U is a neuropeptide, which is expressed in the gastrointestinal, central nervous system and genitourinary[1]. It plays a major role in the contractile activity on smooth muscle. Various other functions of the peptide include: controlling the blood flow and ion transport in the intestine, increased blood pressure and regulation of adrenocortical function[2]. In the central nervous system, their functions are not yet clearly stated, but it might include: neuroendocrine control, regualtion of food intake, modulation of dopamine actions and involvement in neuropsychiatric disorders[3].

Two different types of neuromedin U receptors have been identified, which mediates the action of neuromedin U. They belong to the G protein-coupled receptor family, haivng differing expression patterns[4]. They are expressed throughout the body, having diverse but specific roles. Neuromedin U receptor 1, one of the subtypes, is found mainly in the gastrointestinal tract, whether neuromedin U receptor 2 is found in the central nervous system[5,6]. NmUR1 is involved in the regulation of feeding and energy homeostasis[7-9] and believed to be one of the links between stress and cancer. It is also involved in the pathophysiology of various immune mediated inflammatory diseases like asthma. It plays crucial in maintaining the biological clock, in the regulation of smooth muscle contraction in the gastrointestinal and genitourinary tract, and in the control of blood flow and blood pressure[10-13]. Drug desiging selective towards these receptors, might help in identifying their pathophysiological roles in the diseases related to them.

Homology modelling is an alternate tool helps in predicting the three-dimensional conformation of a protein, when only the sequence data of the protein is available. Due to the enormous amount of time required to prepare protein for crystallization using experimental process such as protein expression, purification and crystallization, the number of protein structures resolved experimentally lags behind the sequence data available[14]. Homology modelling can provide as a tool for the experimental procedures in finding the structure of the protein in a rather short time. In this study, we have developed three-dimensional models of neuromedin U neuropetide based on homology modelling and validated them. The developed models were then docked with the models of NmUR1. The crucial residues of the

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binding interaction identified using this study could be helpful in identifying novel and potent drugs for the treatment of the diseases related to NmUR1.

2. Material and Methods

2.1. Homology Modelling

The amino acid sequence of the human neuromedin U neuropeptide (accession No: P48645) was retrieved from the Uniprot database. Using the modelling platforms, QUARK, an online server for ab initio protein structure prediction[15], was used to model the three dimensional structures of human neuromedin U protein. QUARK aims to model the correct protein three dimensional structure from amino acid sequence, using a computer algorithm which includes ab initio protein folding and protein structure prediction. They develop models from small fragments (1-20 residues long) by replica-exchange Monte Carlo simulation using an atomic-level knowledge-based force field. It was the No 1 ranked Free-modeling (FM) online server in CASP9 and CASP10 experiments. As the QUARK uses no global template information, it is suitable for proteins without any homologous templates.

10 models were developed using the QUARK server. The predicted models were validated using Ramachandran plots, Verify3D, ERRAT plots and ProSA. RAMPAGE web server was used to plot the Ramachandran plots[16]. Ramachandran plot provides a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure, which identifies the sterically allowed regions for these angles. Verify3D determines the compatibility of the predicted model with its own amino acid sequence by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results to good structures[17]. ERRAT plots are plotted as a function of the position of a sliding 9-residue window[18]. The error function is based on the statistics of non-bonded atom-atom interactions present in the structure. ProSA-web, a interactive web server is used to identify the errors in three-dimensional structure of the protein[19].

2.2. Protein-protein Docking

To perform protein-protein docking of Neuromedin U with the Neuromedin U receptor 1 ClusPro 2.0, a protein-protein docking server was used[20,21]. ClusPro is identified as the best web server to perform protein-protein docking and has performed well in the critical assessment of prediction of interactions (CAPRI)[22,23]. ClusPro works on a correlation method known as PIPER[24] which calculates the docked conformation energy in a grid using fast Fourier transform (FFT) coupled with pairwise interaction potentials. As a result of the more accurate pairwise interaction potential of PIPER, much fewer near-native structures were only retained. The structures were clustered based on the pairwise RMSD as the distance measure and were optimized.

3. Results and Discussion

3.1. Model Validation

The predicted models were validated using various validation techniques. Root mean square deviation

Table 1. Model validation results - RMSD and Ramachandran plot values

Model No	Ramachandran Plot			ProSA Z-Score	ERRAT Overall quality factor	Verify3D (% of the residues had an averaged 3D-1D score >= 0.2)
	Number of residues in favored region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)			
1	84.1	8.6	7.3	-6.47	62.069	82.35
2	86.1	8.6	5.3	-5.96	71.034	86.93
3	81.5	12.6	6.0	-5.50	57.241	88.24
4	77.5	11.3	11.3	-5.90	45.139	81.05
5	85.4	9.3	5.3	-6.01	59.310	87.58
6	86.1	7.3	6.6	-6.96	63.448	89.54
7	80.1	13.9	6.0	-6.03	55.862	86.27
8	84.1	5.3	10.6	-5.49	64.138	84.31
9	76.2	9.3	14.6	-6.13	53.521	98.04
10	80.1	11.3	8.6	-5.32	58.621	92.16

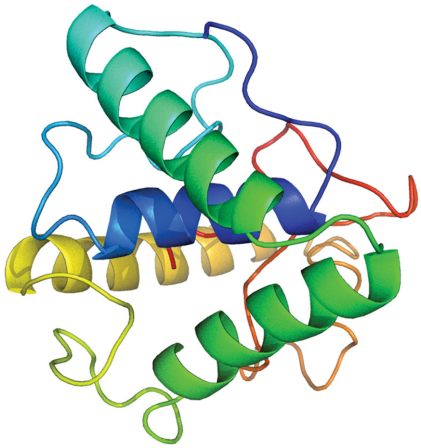

Fig. 1. Best model (Model 2) selected after validation.

(RMSD) of all the predicted models with their respective template was calculated. Ramachandran plot was generated for each model and the number of residues in favourable, allowed and disallowed region was identified. Verify3D and ERRAT plots were developed for the models. Using ProSA web server Z-scores were calculated. The statistics of model validation are represented in the Table 1. Based on the statistics, the model 2 was found to be the best models. Model 2 scored well in all the validation and found to be the most reliable among the developed models (Fig. 1). RC plot and ERRAT plots of the selected models were represented in Fig. 2 and Fig. 3 respectively.

3.2. Molecular Docking of Neuromedin U Receptor 1- Neuromedin U

We have performed protein-protein docking to identify the crucial residues involved in the interaction of the natural agonist, neuromedin U, with the receptor NmUR1. The structure of neuromedin U developed using the QUARK server was used, along with the 3D models of the receptor developed using homology mod-

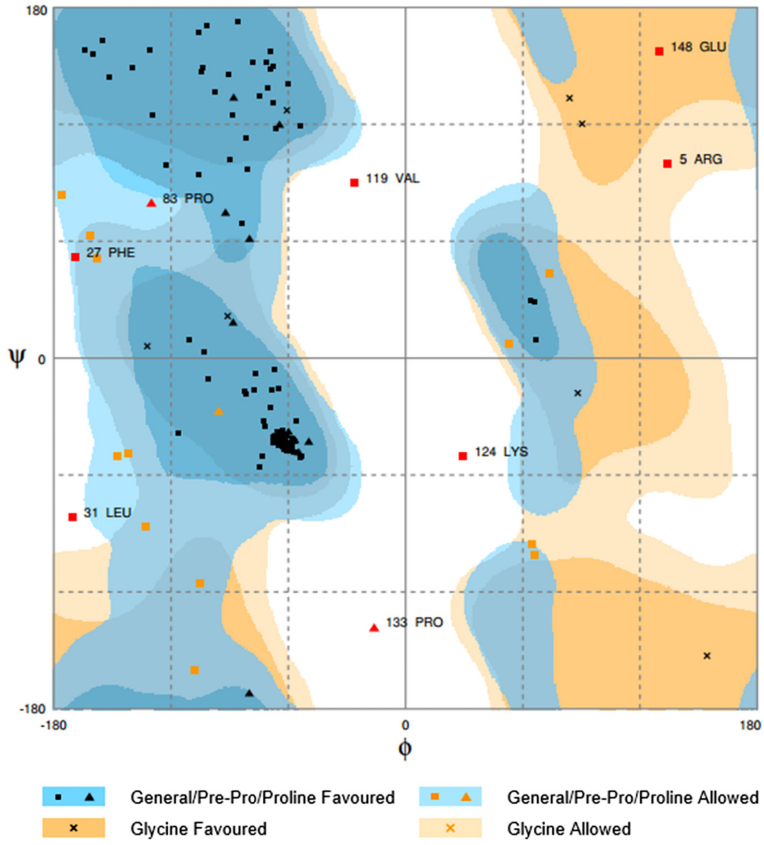

Fig. 2. RC plot for selected model – Model 2.

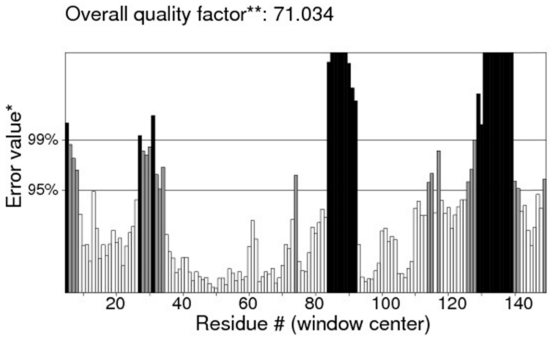

Fig. 3. ERRAT plot developed for the selected model – model 2.
*on the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value

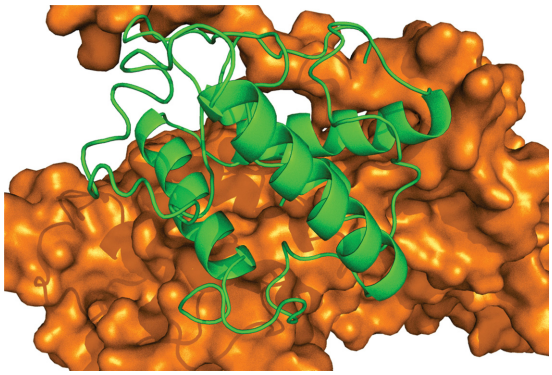

Fig. 4. Binding mode of the native agonist (Neuromedin U) with the receptor (Neuromedin U receptor 1).

eling. CLUSPRO 2.0 server was used to do protein-protein docking, and 24 different clusters of docked complexes were generated. The top cluster consists of 98 members, and lowest energy weighted score was -1161.4. The cluster scores are represented in the Table 2. The top cluster was chosen was studying the interaction between the receptor and the ligand. We have identified the important residues involved in the interaction. Fig. 4 displays the binding mode of the peptide with the receptor.

4. Conclusion

Three dimensional models for neuromedin U were generated using the QUARK web server. Model number 2 was selected as the best model, based on their

Table 2. Cluster Scores developed using ClusPro server

Cluster	Members	Representative	Weighted Score
0	98	Center	-1161.4
		Lowest Energy	-1382.3
1	89	Center	-1087.2
		Lowest Energy	-1332.3
2	85	Center	-1060.4
		Lowest Energy	-1479.2
3	65	Center	-1064.8
		Lowest Energy	-1313.6
4	58	Center	-1045.7
		Lowest Energy	-1391.6
5	55	Center	-1067.2
		Lowest Energy	-1195.1
6	50	Center	-1202.9
		Lowest Energy	-1426.5
7	46	Center	-1228.4
		Lowest Energy	-1305.1
8	37	Center	-1050.9
		Lowest Energy	-1347.6
9	37	Center	-1188.7
		Lowest Energy	-1188.7
10	34	Center	-1099.4
		Lowest Energy	-1407.6
11	32	Center	-1123.0
		Lowest Energy	-1285.9
12	31	Center	-1069.6
		Lowest Energy	-1243.0
13	29	Center	-1048.7
		Lowest Energy	-1362.6
14	27	Center	-1051.0
		Lowest Energy	-1144.9
15	27	Center	-1154.9
		Lowest Energy	-1205.5
16	21	Center	-1046.3
		Lowest Energy	-1243.4
17	19	Center	-1141.1
		Lowest Energy	-1141.1
18	19	Center	-1188.7
		Lowest Energy	-1188.7
19	15	Center	-1157.3
		Lowest Energy	-1228.5
20	14	Center	-1196.8
		Lowest Energy	-1196.8
21	13	Center	-1070.5
		Lowest Energy	-1108.2
22	10	Center	-1199.6
		Lowest Energy	-1199.6
23	8	Center	-1058.9
		Lowest Energy	-1123.4
24	4	Center	-1097.3
		Lowest Energy	-1108.1

RMS deviation, Ramachandran plot, ProSA, ERRAT plot and Verify3D values. Based on the results after model validation, it is found that all the generated models are similar and the structures are reliable. The selected was then docked with a homology model of Neuromedin U receptor 1. The resultant docked complex could help in identifying the crucial residues involved in the receptor-ligand complex formation.

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