In silico interaction analysis of motilin hormone with its receptor

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ABSTRACT

The gastrointestinal system produces diverse peptide hormones, including a 22-amino acid long human motilin which is essential for initiating inner digestive migratory contractions interacts directly with receptors on gastrointestinal smooth muscle cells. This interaction is a focal point for potential drug targeting in gastrointestinal disorders. The modulation of gastrointestinal motility through motilin-receptor interplay presents a promising avenue for therapeutic interventions. This study aims to elucidate the molecular interactions between a motilin and its cognate receptor. The 3D structure of motilin hormone was obtained in purified form from the PDB database, while the unavailable motilin receptor structure was homology-modeled using I-TASSER. Validation and quality checks were performed using ERRAT and Procheck while physiochemical properties were predicted with ProtParam and both, motilin and its receptor, underwent energy minimization on Galaxy Refine for subsequent docking studies on Cluspro online server. Next docking was validated with an RC plot, and analyzed for interaction studies on PDBsum and PDBePisa.. The motilin receptor model demonstrated structural stability, supported by an 86% ERRAT score and 95.5% of residues within the favored regions of the Ramachandran plot. Docking analysis yielded a stable complex with 7 hydrogen bonds and 1 disulphide linkage, validating the study's integrity. This in silico studies of motilin with its receptor, provides a reliable foundation for further functional, structural, and therapeutic investigations, suggesting potential avenues for designing motilin analogues to design motilin-related disorders.

Keywords: Motilin, in silico analysis, GI disorder, docking

INTRODUCTION

The endocrine system plays a crucial role in maintaining homeostasis within the human body, orchestrating a symphony of chemical messengers known as hormones. Among these, gastrointestinal hormones hold particular significance, being peptides released by both endocrine cells and neurons in the portal system (1). These hormones play a pivotal role in regulating various functions of the gastrointestinal system. They act as specialized communicators, targeting specific cells to control smooth muscle contractions that facilitate the movement of food through the digestive tract. This intricate coordination ensures the proper digestion and absorption of nutrients, highlighting the indispensable nature of gastrointestinal hormones in the seamless functioning of the digestive processes. (2, 3).

The human motilin hormone is a 22-amino-acid peptide hormone, (4) which is involved in regulating gastrointestinal motility (5) and gall bladder emptying (6) of the gastroduodenal tract by cyclic increase of motilin in circulating plasma. Motilin regulates the phase III contraction of the migrating motor complex in the upper gut (7). It is a motor pattern that takes place in the human gastrointestinal tract during the interdigestive stage or varies in each phase of MMC (migrating motor complex) (8). Motilin is produced by the M cells, found in the portion of the small intestine duodenum that encourages the smooth muscles of the gastrointestinal tract to contract (9). Bile and stomach acids are released into the duodenum, these two primary physiological regulators take control of endogenous motilin which is released in fasting state. This regulation has a role in the modulation of the migrating motor complex, which in turn controls the release of GI hormones, the transmission of hunger signals, and the absorption of nutrients (10). The NH_2 terminus of motilin, which is involved in the incorporation of its receptor, is stabilized by the COOH-terminal of the protein, or the C-terminus of motilin. The motilin receptor (MLN-R), a G protein-coupled receptor (GPCR), mediates the effect of motilin. Motilin is expressed in the GI mucosa while its cognate receptor (MW:41 kDa, amino acid: 347) is found on enteric neurons, smooth muscle cells of the GI tract and central nervous system (CNS). The Motilin receptor's expression and distribution are regulated by various factors, including hormones, neurotransmitters, and dietary factors (9).

The absence or alteration of any hormone in its normal functioning is associated with some disorder. In the case of motilin deficiency, disruptions in physiological function may manifest, potentially leading to constipation, particularly in children (10). On the other hand, elevated

plasma motilin levels have been observed in individuals with diabetes following acute pancreatitis (11). This suggests a potential link between motilin and the development of diabetes in this specific clinical context.

The use of bioinformatics tools in this study holds paramount significance as it enables an in-depth exploration of the motilin hormone and its receptor interaction at a molecular level. Bioinformatics tools provide a powerful and efficient means to analyze complex biological data, offering insights into the structural dynamics, binding affinities, and potential functional implications of the motilin-receptor interaction. Limited research articles exist on the interaction studies between motilin and its receptor, highlighting a gap in understanding this crucial physiological mechanism. Recognizing this, the current study aims to in silico investigations of interactions between motilin hormone and its cognate receptor. Which could reveal the intricacies of their interaction, shedding light on the molecular dynamics and potential binding sites. By delving into the molecular aspects of motilin and its receptor, this in silico study not only contributes valuable insights into the underexplored realm of gastrointestinal physiology but also holds the promise of offering a foundation for future experimental work.

MATERIAL AND METHODS

Human Motilin hormone 3D structure retrieval

The purified 22 amino acid long, motilin hormone 3D structure was retrieved from Protein Data Bank (PDB) (https://files.rcsb.org/download/1LBJ.cif.gz) (Accession No: 1LBJ).

Construction of motilin Receptor

The 3D structure of the motilin receptor wasn't available on the protein database (PDB) so its FASTA sequence (412 amino acid residues) was retrieved from the National Center for Biotechnology Information (NCBI) database https://www.ncbi.nlm.nih.gov/protein/?term=motilin+receptor. Accession (NP_001498.1).

Homology modeling of motilin receptor

While performing the homology modeling, the FASTA sequence of the receptor was provided as an input in the I-TASSER (Iterative Threading Assembly Refinement) server (https://zhanggroup.org/I-TASSER/) for modeling of the 3D structure of motilin's receptor.

Refinement and validation of motilin receptor:

For quality assessment and validation, online server of SAVES v6.0 tool package (https://saves.mbi.ucla.edu/) was used (12). Thus validation and stability of all modeled protein

was determined by the Ramachandran plot (13) by the PROCHECK program. VERIFY3D evaluated the compatibility of an atomic model (3D) with its amino acid sequence. Moreover, the overall quality factor was evaluated through ERRAT software (14) (15) and ProSA-web was used for a quality score based on a specific input structure and displayed it in the context of all known protein structures, facilitating and detecting scores outside the range of native proteins. (https://doi.org/10.1093/nar/gkm290). Next for refinement, the Galaxy WEB server (https://galaxy.seoklab.org/index.html/) was used for the loop or terminal sections improvement through ab-initio modeling that predicts protein structure from sequence using template-based modeling (16).

Physiochemical properties of hormone and receptor structure

The ProtParam tool was used to examine the physiochemical properties of the motilin hormone https://web.expasy.org/protparam/, which includes its theoretical (pI), amino acid composition(%), molecular weight, instability index, atomic composition, negatively charged amino acids (Asp + Glu), positively charged amino acids (Arg + Lys) and GRAVY (grand average of hydropathicity) (17, 18).

Molecular docking and interaction

The molecular docking technique is used to simulate the atomic-level interaction between receptor and ligand molecules, enabling us to characterize how a hormone behaves at the binding site of its target receptor protein. The docking produces a target-ligand complex that resembles the "native" complex in the biological system to understand fundamental biological processes in a better way. (19, 20). For current study the molecular docking was performed on ClusPro, an online server for automatic computational docking of protein structures (https://cluspro.org/help.php). ClusPro runs three computational steps including rigid body docking, root-mean-square deviation (RMSD) clustering, and the refinement of selected structures using energy minimization. (21, 22). The interactions between motilin and its receptor were analyzed on PDBePISA (https://www.ebi.ac.uk/pdbe/pisa/) and PDBsum online servers which can predict the salt bridges, hydrogen bonding, surface interface area or intermolecular protein–protein interactions, interactions among the residues or binding energy between molecules of the docked complex. Furthermore, the protein binding energy (PRODIGY) webserver was used for calculating the affinity for binding (G) and the dissociation constant (Kd) of the complexes to accurately predict the binding strength of the protein complexes (23).

Results and discussion

Sequence and 3D structure retrieval of motilin hormone and its receptor

The 3D structure of the Human Motilin hormone in purified form was retrieved from the Protein Data Bank (PDB) (Figure 1). Motilin receptor, which was not available in 3D structure, was constructed by using its FASTA sequence, retrieved from the National Center for Biotechnology Information (NCBI) database.

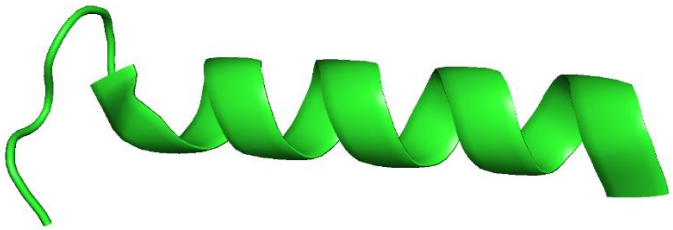

Figure 1: Human motilin hormone 3D structure

Homology modeling and refinement of motilin receptor

For preparing the 3D structure of the receptor homology modeling, the FASTA sequence of motilin receptor was submitted to I-TASSER software which predicted 5 distinct 3D models. The model with the highest C-score of -1.27 was chosen because it shows higher confidence (typically the range of C-score range between -5 to 2) as shown in figure 2. This model had a TM score of 0.56+-0.15 and an RMSD of 9.8+-4.6Å which tells the accuracy of the model (Figure 2) (24). Thus, refinement of the model was performed by the Galaxy WEB server which provided 5 models among them the top structure with the highest TM and RMSD score was chosen for further quality assessment and validation. Before refinement, the Ramachandran plot showed the favorable region residues which was 82.3%, residues in additionally allowed region were 12.2%, and in disallowed region was 3.5%.

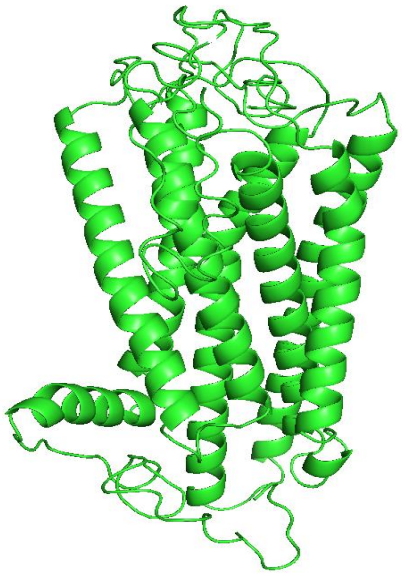

Figure 2: Motilin hormone receptor 3D structure

Human motilin receptor validation

The refined receptor was validated by RAMPAGE which provided the Ramachandran plot that represents the residue validation on the basis of φ (phi) and ψ (psi) dihedral angles of amino acids residues in the favored region, allowed region, or outlier region (25). After refinement the selected model showed 84.0% residues in the most favorable area, 11.0% in additional allowed areas, 1.5% generously allowed regions, and 3.5% in disallowed regions shown in figure 4(A). Furthermore, for quality check, ERRAT gave the overall quality factor that detects the error in protein regions leading to the random distribution of atoms. The selected model has an 83.4184 quality factor with ERRAT (26) (27) as shown in figure 4(B). For further evaluation of the model the proSA-web server indicated the Z -score which was -4.43 (figure 3 (A and B). Finally, the model was checked by Verify-3D which revealed that 65.05% of the amino acid residues had average score >= 0.1 in the 3D-1D profile for scoring the compatibility of amino acid residues sequence in the model protein (24) (27).

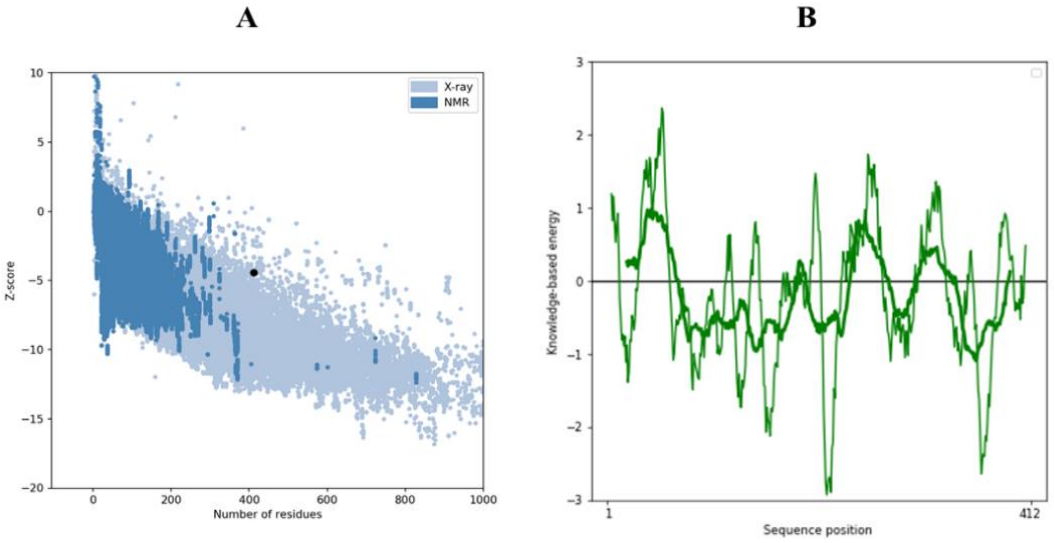

Figure 3: (A) Z-score of motilin receptor construct. (B) energy distribution plot of residues

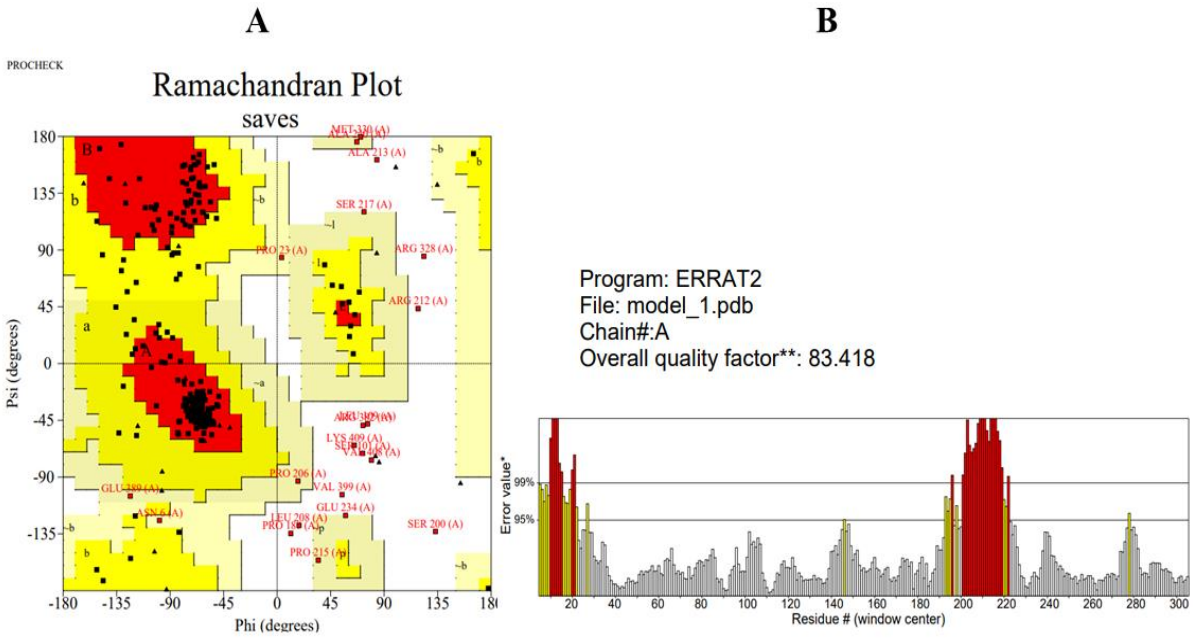

Figure 4. (A) Ramachandran plot of motilin recptor. (B) ERRAT quality graph.

Physiochemical properties of motilin receptor

The ProtParam server was used to calculate the physicochemical properties of the motilin receptor in Table 2. The molecular weight of the protein was 45344.30 with more basic amino acids (Arg + Lys) =44 than acidic (Asp + Glu) = 24 which impart protein molecule an alkaline

nature with a theoretical isoelectric point of pI= 9.97. The Extinction coefficient of modeled protein was 280 M-1 cm-1, an important protein interaction or the estimated half-life was 30 hours (mammalian reticulocytes, in vitro) and the instability index was computed to be 52.20. The estimated Aliphatic index= 103.91 tells the thermostability of the protein and the Grand average of hydropathicity (GRAVY) = 0.291 shows that protein was soluble in water (28).

Table 1: Physiochemical properties of motilin receptor contruct

Physiochemical properties	**Values**
Number of amino acids	412
Theoretical pI	9.97
Molecular weight	45344.30
Instability index	52.2
Aliphatic index	103.91
Total positively charged amino acids	(Arg + Lys): 44
Total negatively charged amino acids	(Asp + Glu): 24
Grand average of hydropathicity (GRAVY)	0.291
Predicted half-life	30 hours (*E coli*, in vivo)
Coefficient of extinction (in M^{-1} cm^{-1} at 280 nm)	71850

Molecular docking of motilin hormone with cognate receptor

A molecular docking model involves two or more molecules interacting with each other, which predicts the 3D structure of complex depending upon the binding properties of hormone and its receptor. ClupPro 2.0 webserver works on rigid body docking method with different weighted scores of the center and the lowest energy. The selected model was a 149 balanced coefficient member whereas its center-weighted score is -960.9 and the lowest energy score is -960.9 (Table 2). The center score indicates the highest structure energy in neighboring structures and the weighted score of lowest energy indicates the lowest energy structure in that cluster. After docking, the complex was again submitted to Ramachandran plot analysis for docking validation which showed the residues in favourable region 81.7%, additional allowed region contained 13.0%, generously allowed region contained 1.9% and disallowed region contained 3.3% residues.

Table 1: Balanced coefficient score of dock complex.

Cluster	Members	Representative	Weighted Score
0	267	Center	-919.2
		Lowest Energy	-945.3
1	162	Center	-923.5
		Lowest Energy	-923.5
2	138	Center	-870.5
		Lowest Energy	-870.5
3	125	Center	-841.7
		Lowest Energy	-918.7
4	51	Center	-788.2
		Lowest Energy	-841.1
5	41	Center	-850.8
		Lowest Energy	-877.1
6	41	Center	-803.4
		Lowest Energy	-873.1
7	32	Center	-824.0
		Lowest Energy	-824.0
8	32	Center	-810.6
		Lowest Energy	-810.6
9	30	Center	-868.6
		Lowest Energy	-868.6

Interaction analysis

Although dozens of tools based on analytical or empirical approaches are available, but computational estimation of binding energy in the protein-protein interface is a complex problem. The effectiveness of each tool varies depending on the specific protein, and we cannot identify a single module that provides a trustworthy quantification. The docked residues were analyzed for interactions by using online servers of PDBePISA and PDBsum which evaluates the interface residues, interface surface, salt bridges, hydrogen bond, and $\Delta G^{int.}$ (Solvation Energies). The interactions between the docked complex showed a total 7-interactions

mediated by 1 salt bridge in residues was Arg^{65} of chain A and Glu^{15} chain B and 6 hydrogen bonds between the residues Arg^{65}, Arg^{156}, Arg^{156}, Ala^{213}, Arg^{373}, Arg^{373} of chain A motilin receptor and Glu^{15} , Phe^{1}, Phe^{1}, Glu^{17}, Gln^{22}, Gln^{22} of chain B motilin hormone (Figure 5 and 6) (23). Another sever PDBePISA which predicted the binding affinity (solvation energy) of the docked complex showed a score of -12.5kcal/mol with interference area between the chains was 872.5$Å^2$. The complex of motilin hormone and receptor had a binding affinity -8.0kcal/mol^{-1} at 25-degree temperature by prodigy online web server.

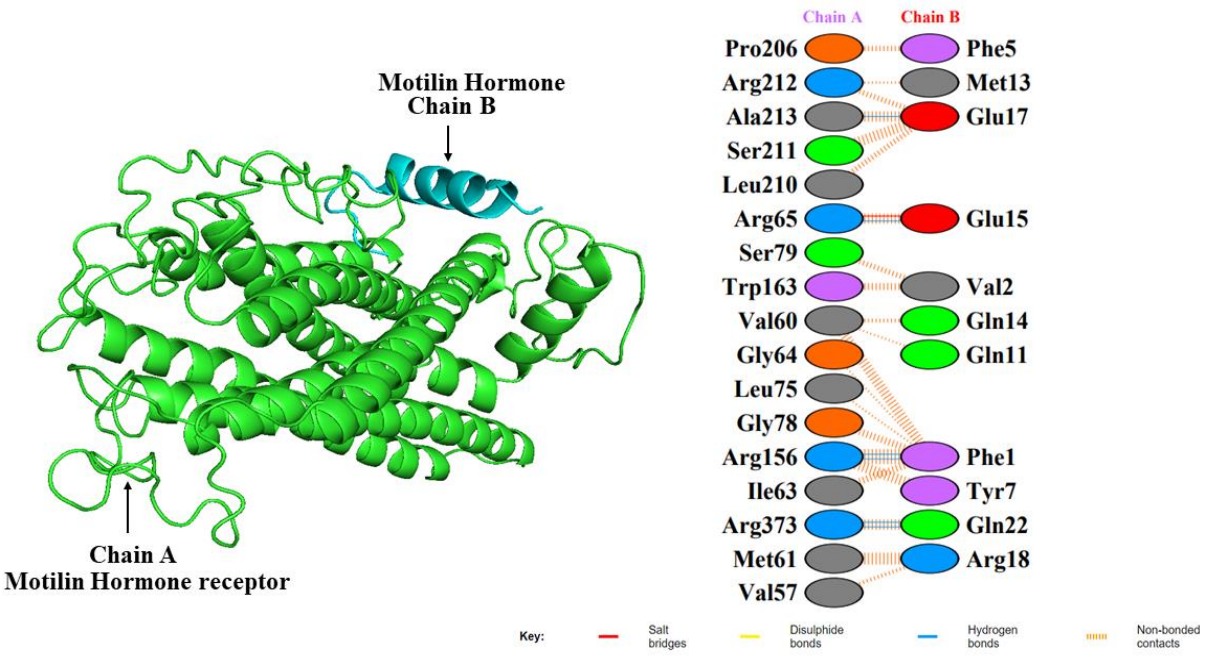

Figure 5: Residue interactions between Motilin Hormone and receptor was analyzed by PDBsum.

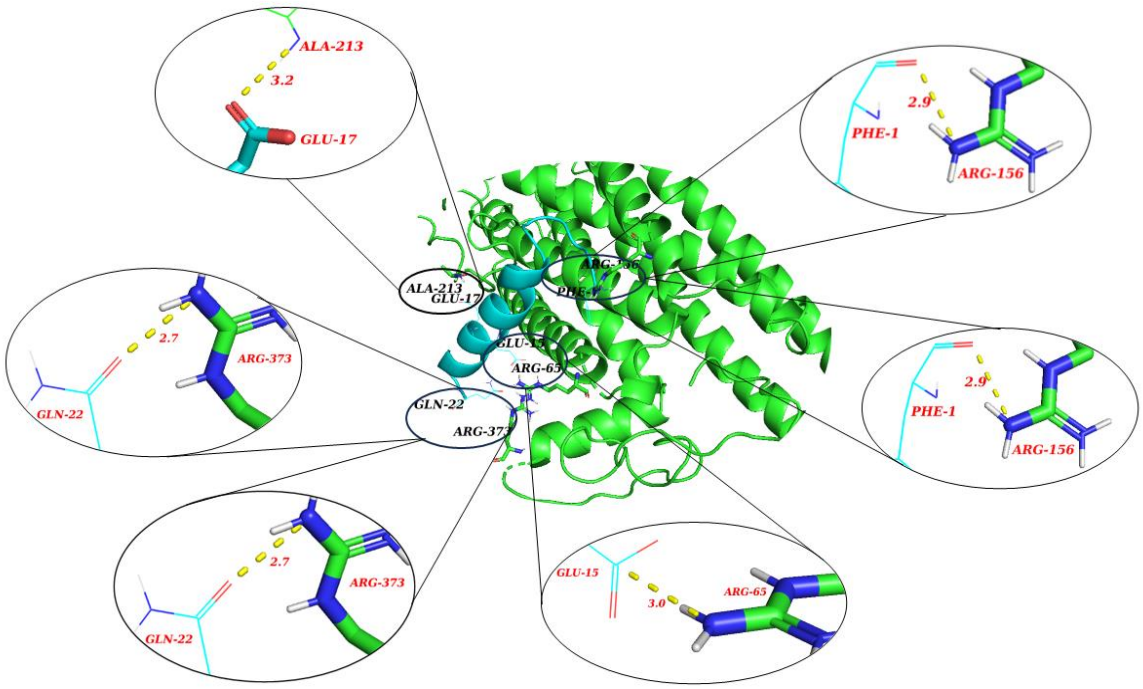

Figure 6: Interacting residues of motilin hormone with its receptor.

Conclusion

In conclusion, this comprehensive in silico study has successfully elucidated the molecular interactions between motilin and its cognate receptor, offering valuable insights into the essential role of motilin in initiating inner digestive migratory contractions. The 3D structure of motilin was obtained from the PDB database, while the motilin receptor structure was effectively homology-modeled using I-TASSER, demonstrating structural stability and reliability with high validation scores. The subsequent docking studies on Cluspro revealed a stable complex between motilin and its receptor, supported by multiple hydrogen bonds and a disulphide linkage. The 100ns Gromacs simulation further confirmed sustained stability and provided a detailed analysis of intricate hydrogen bonds and salt bridges. This in-depth exploration not only enhances our understanding of the motilin-receptor interplay but also establishes a robust foundation for future functional, structural, and therapeutic investigations. The findings suggest promising avenues for drug targeting in gastrointestinal disorders, potentially paving the way for the design of motilin analogues to address motilin-related disorders.

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