

Short Communication

VIRTUAL TARGET CONSTRUCTION FOR STRUCTURE-BASED SCREENING IN THE DISCOVERY OF HISTAMINE H₂ RECEPTOR LIGANDS

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ABSTRACT

Objective: This study aimed to develop validated targets to be employed in structure-based virtual screening (SBVS) to discover ligands for the human histamine H₂ receptor (hHRH2).

Methods: The virtual targets construction was initiated by homology modeling with the reference compound ranitidine as the ligand followed by 100 ns molecular dynamics (MD) simulations. During MD simulations, the snapshot with the lowest value of the free energy of binding was selected for further validation by re-docking simulations. All simulations were performed in YASARA-Structure.

Results: The research presented here resulted in one validated target for the SBVS. Additionally, by employing a clustering module in MD simulations analysis in YASARA-Structure, more than ten different virtual targets are also available for further uses.

Conclusion: The virtual targets resulted in this research offer possibilities to construct valid SBVS protocols to identify ligands for the hHRH2.

Keywords: Histamine H₂, Ranitidine, Homology modeling, Molecular docking, Molecular dynamics

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Histamine H₂ receptor (HRH2) plays an essential role in regulating gastric acid, the secretion of which facilitates protein digestion [1]. The receptor contributes to the absorption of micronutrients and certain drugs, eliminating ingested microorganisms and preventing bacterial overgrowth and enteric infections [2]. Receptor therapy contributes in treating peptic ulcers and gastro-oesophageal reflux disease (GORD) [1]. This disease is a global health epidemic, with billions of people suffering from gastric disorders to gastric cancer [3].

Due to these significant roles, it is necessary to perform research regarding the human HRH2 (hHRH2) as the target of drug discovery projects [4]. One of the effective methods in the computer-assisted discovery of new drugs is structure-based virtual screening (SBVS) [5]. The approach is efficient for the design, discovery, and optimization of bioactive compounds [6] and is capable of generating directions for further drug development [7], projecting the ligand binding pose, estimating its affinity for the target protein [8], modeling accurate structures, and predicting activity precisely [9].

The main instrument used in this study was a hardware with Intel® Xeon 2680 V3 as the processor, Nvidia Quadro M4000 as the video graphic card, 500Gb SSD, 64Gb ECC Registered RAM. The operating

system was Ubuntu 20.04.1 LTS, and the main software was YASARA-Structure 20.1.2410 [10]. The primary material used in this research was the crystal structure of the human histamine H₁ receptor (hHRH1) [11] (PDB ID: 3RZE) obtained from <https://www.rcsb.org/structure/3RZE>. The reference compound structure, i.e., ranitidine was taken from <https://www.go.drugbank.com/drugs/DB00863>, while the hHRH2 target sequence was obtained from <https://www.uniprot.org/uniprot/P25021>.

Homology modeling hHRH2 were run with YASARA-structure through the module "Homology modeling". The hHRH1 crystal structure was used as the template, which consisted of 397 amino acid residues. Alignment correction and loop modeling were performed by predicting secondary structures for target sequences using PSI-BLAST, then they were included in the PSI-Pred secondary structure prediction algorithm [12]. Five homologous models resulted from the homology modeling were then sorted by Z-score. Subsequently, YASARA-structure combines the best parts of the five models to get a hybrid model with increased accuracy beyond each contributor, shown in table 1. A total 327 of the 397 target residues (82.4%) were aligned with the template residues, the order identity was 24.5%, and the sequence similarity was 45.0%.

Table 1: Result of the hybrid model obtained the following quality Z-scores

Check type	Quality Z-score	Comment
Dihedrals	0.034	Optimal
Packing 1D	-0.735	Good
Packing 3D	-1.437	Satisfactory
Overall	-0.950	Good

The selected receptor homology model was used as the target for ranitidine docking, performed 1,000 times, and iterated 25 times using the VINA method. Molecular docking simulations were run with YASARA-Structure by using the dock_run.mcr command which is provided default by YASARA-Structure. Thirteen suggested ligand-receptor complexes were ordered based on the best free energy of binding. Since the molecular docking simulation provided visual information of the protein-ligand interactions [13], visual inspection played an important role in the pose selection. According to Shin *et*

al., Asp98 in hHRH2 was fundamental for binding of essential antagonists [14]. Therefore, based on the visual inspection, the docking pose of number 12 was selected with the binding energy of -7.290 kcal/mol.

Molecular dynamics (MD) simulations of the complex embedded in the membrane were then carried out to obtain an overview of the most stable ligand-receptor bond pose [16], were run with YASARA-Structure by employing the md_runmembrane.mcr command which

is provided default by YASARA-Structure. The MD simulations were carried out with the following settings: The force field AMBER14, pH 7.4, temperature 298K, pressure 1 bar. Simulations were run at up to 100 ns, and simulation snapshots were stored at 100 ps intervals. The snapshots were then analyzed to examine the structural stability and the free energy of binding.

The MD simulations showed that the RMSD values remained constant since 5 ns. (fig. 1. a). Delta RMSD values were calculated every 5 ns, and the data shows that most of the times the values are less than 1 Å (fig. 1. b), which indicates that the system was

stable. According to Liu *et al.*, stability is achieved if RMSD values' deviation in the last 5 ns in the MD simulation is less than 1 Å [15].

The free energy of binding in MD simulation is calculated using VINA embedded in YASARA-Structure (fig. 2). The most stable system was formed at the 838 picosecond time interval with the free energy of binding value of -9.654 kcal/mol. This system was selected and analysed further as validated virtual target (fig. 3). Thermodynamically, the best receptor-ligand interactions occur if the resulting complex has lower potential energy [16]

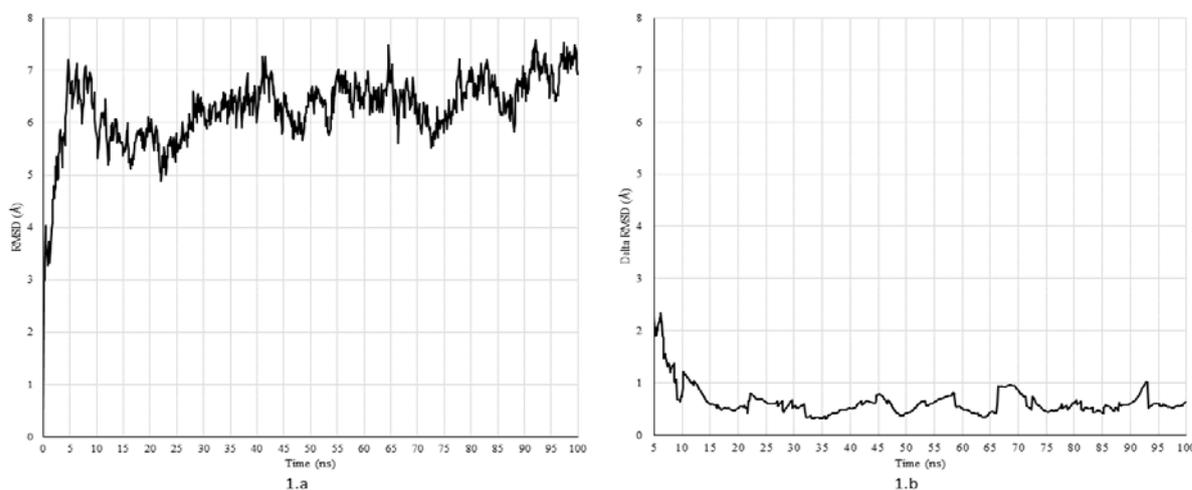


Fig. 1: a RMSD (Å) vs. Time (ns) graph, 1. b. Delta RMSD (Å) vs. Time (ns) graph

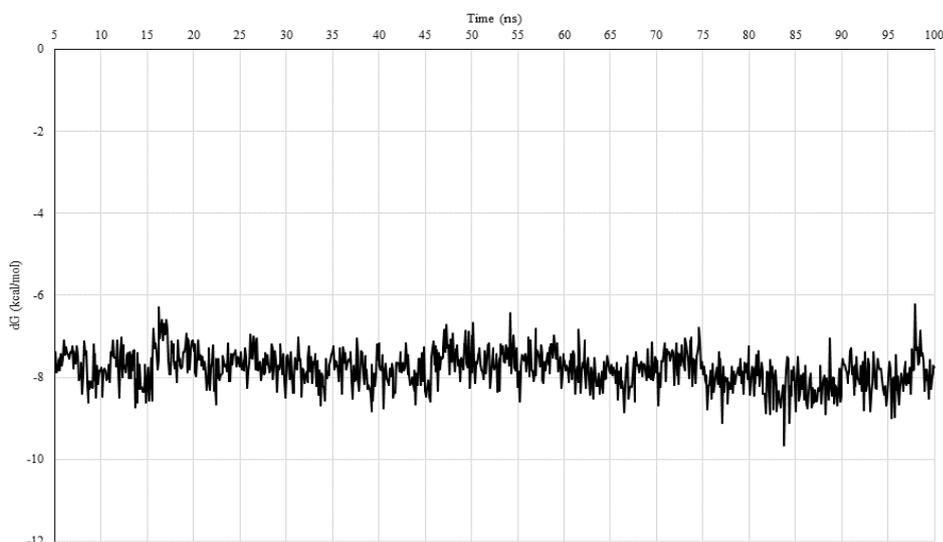


Fig. 2: The free energy of binding (kcal/mol) vs time (ns) graph

The validation was carried out by re-docking the receptor-ligand complex at snapshot 838 for 1000 times with 25 iterations. The ligand pose resulting from re-docking was compared to the reference ligand pose and the RMSD values were calculated [17]. The simulation of repeated docking 1000 times resulted in all RMSD data generated having a value of less than 2 Å, indicating that the resulting confidence level satisfies the rule [18]. This value is the first step in developing a

quality structure-based virtual screening protocol [17] to identify histamine H₂ inhibitors. Subsequently, in order to have 10 cluster systems, clusterization of the molecular dynamics simulation results was carried out by using 3.6 Å as the minimum RMSD of snapshots between clusters. The clusterization was intended to provide more virtual targets for further development of structure-based screening in the discovery of hHRH2 ligands.

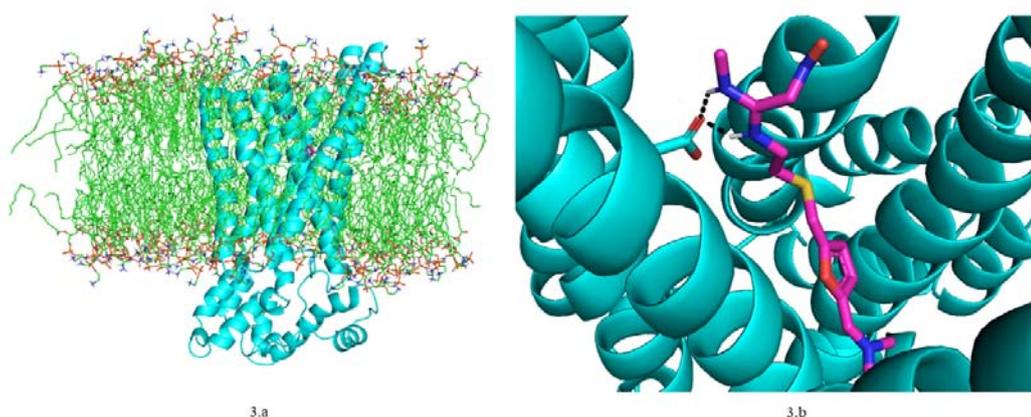


Fig. 3: a The most stable system at the 838 picosecond time interval, 3. b Visual inspection of the interaction of ranitidine to Asp98

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AUTHORS CONTRIBUTIONS

G. N. conducted all the computational simulations, and E. P. I supervised the work. G. N. initiated the original draft of the manuscript. E. P. I reviewed and edited the manuscript. All authors have given approval to the final version of the manuscript.

CONFLICTS OF INTERESTS

All authors have none to declare.

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