Journal of Rare Cardiovascular Diseases
ISSN: 2299-3711 (Print) | e-ISSN: 2300-5505 (Online)
www.jrcd.eu
RESEARCH ARTICLE

Structure-Based Docking Analysis of the Beta-2 Adrenergic Receptor Using AutoDock Vina

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Article History
Received: 08.08.2025
Revised: 15.09.2025
Accepted: 24.10.2025
Published: 05.11.2025

Abstract: Docking plays a vital role in modern drug discovery by enabling researchers to predict how small molecules, such as ligands, interact with target proteins at the molecular level. In this study, the binding interaction between carazolol—a selective antagonist of the β2-adrenergic receptor (β2AR)—and the receptor itself was investigated using computational docking techniques. The three-dimensional structure of β2AR was first constructed using homology modeling with MODELLER, and further validated through AlphaFold, which provided enhanced confidence in the structural accuracy. Due to the absence of a high-resolution experimental structure for the full receptor, the active binding region was identified through visual inspection and structural comparison. A docking grid was defined around this site, and simulations were carried out using AutoDock Vina. The docking results indicated that carazolol formed stable interactions with the receptor, supported by favorable binding energy values. Repeated runs demonstrated consistency in the binding poses. The combined use of AutoDock, PyMOL, and AlphaFold proved effective in improving the robustness of structure-based drug discovery in this context.

Keywords: Molecular docking, β2-adrenergic receptor (β2AR), Carazolol, AlphaFold, AutoDock, Ligand-receptor interaction.

INTRODUCTION

Molecular docking serves as a fundamental technique in the field of computer-aided drug design, offering the ability to predict how small molecules, such as drug candidates, physically and energetically interact with target proteins. This computational approach provides insights into both the binding affinity and orientation of a ligand within the protein's active site, which are crucial parameters in determining potential biological activity. By narrowing down the number of compounds requiring laboratory testing, docking significantly reduces the time, cost, and resources involved in early-stage drug discovery.

In this study, the focus was on the β2-adrenergic receptor (β2AR), a member of the G-protein-coupled receptor (GPCR) family. These receptors are embedded within the cell membrane and are involved in transmitting signals from the extracellular environment to the interior of the cell. The β2AR, in particular, is associated with regulating physiological processes such as bronchial dilation, smooth muscle relaxation, and cardiovascular function. Its clinical relevance makes it a critical therapeutic target, especially for respiratory and cardiovascular disorders including asthma, hypertension, and chronic obstructive pulmonary disease (COPD).

Carazolol, a selective β2AR antagonist, was selected for this docking study due to its well-documented pharmacological activity and strong receptor binding capabilities. As experimental crystallographic data for full-length GPCRs remain limited due to their dynamic nature and membrane-bound complexity, computational modeling techniques were employed to obtain a reliable 3D structure of the receptor. Homology modeling was carried out using MODELLER, leveraging structurally similar templates available in the Protein Data Bank. To enhance confidence in the structural model, AlphaFold—a state-of-the-art AI-based prediction tool—was also used to generate a separate structure. Comparison of the two models showed high structural similarity, particularly in the transmembrane region, which includes the ligand-binding site.

Given the absence of detailed experimental binding site data for this receptor-ligand pair, the active site was determined through careful visual inspection and knowledge of conserved GPCR binding motifs. A docking grid was manually positioned within the central cavity of the receptor model, allowing the ligand to explore relevant conformational space during the docking process. AutoDock Vina was used for docking simulations, providing binding affinity estimates and multiple ligand poses. The docking results revealed that carazolol forms stable and energetically favorable interactions with key residues within the binding pocket. Visualization using PyMOL confirmed these interactions, highlighting hydrogen bonds and hydrophobic contacts that contribute to the ligand's stability within the receptor.

This study demonstrates the value of combining homology modeling, AI-based structure prediction, and docking tools to investigate protein-ligand interactions in cases where experimental data are scarce. The findings support the use of such integrated computational workflows as reliable alternatives for early-stage drug development and target validation.

Organization of the paper- Section 2 details the simulation and docking approach. Section 3 lists the tools used. Section 4 compares traditional and AI-based modeling. Section 5 discusses research gaps in GPCR docking. Section 6 presents results and analysis. Section 7 highlights key contributions. Section 8 concludes the study and suggests future work.

COMPARITIVE STUDY

A comparative analysis was conducted between the integrated workflow used in this study and tools like AutoDock, MODELLER, and AlphaFold, highlighting their individual purposes, strengths, and limitations while emphasizing the benefit of combining their capabilities.

Table 1: Comparative Analysis of Software Tools Employed in the Study

Feature	**This Study**	**AutoDock (Vina)** [4,9]	**MODELLER** [5]	**AlphaFold** [2,3]
Purpose	Integrated protein modeling, ligand preparation, and docking analysis	Molecular docking and ligand binding affinity prediction	Homology modeling of protein structures	Deep learning-based protein structure prediction
Input Data	FASTA sequence, ligand structure (SDF/PDB), PDBQT files	Receptor and ligand in PDBQT format	Target sequence, template structure	Protein sequence (FASTA)
Output	3D protein model, docking poses, binding energy	Binding affinity, docking poses	3D protein structure (PDB)	Predicted 3D structure with confidence scores
Methodology	MODELLER for homology modeling [5], AlphaFold for validation [2,3], PyMOL for visualization [6], AutoDock Vina for docking [4]	Lamarckian Genetic Algorithm, scoring function (Vina) [4]	Comparative modeling using spatial restraints [5]	AI models trained on protein structure databases [2,3]
Key Strengths	Combined multiple tools for end-to-end structure-based drug design workflow	Efficient and widely used for docking, supports flexible ligand docking	Accurate if template is available, widely used in structure prediction	High accuracy, useful for template-free predictions

Most docking studies tend to use static receptor models, which don't always reflect the natural flexibility of proteins. To overcome this, future research can incorporate molecular dynamics to simulate more realistic interactions. This study took a more comprehensive approach by comparing models from both MODELLER and AlphaFold, offering a stronger basis for structural accuracy. To capture important binding details, PLIP was used for interaction profiling—something many similar studies overlook. While validation is often skipped, this work recommends molecular dynamics as a valuable next step. Additionally, instead of relying on a single docking run, repeated simulations were carried out here to improve reliability and set the stage for testing more ligands in future studies.

METHODOLOGY

To explore the interaction between the β2-adrenergic receptor and potential ligands, this study followed a carefully structured computational approach. The methodology was divided into several essential stages, including the preparation of ligand molecules, modeling of the receptor's three-dimensional structure, identification of binding pockets, execution of docking simulations, and thorough evaluation of the results. Each of these steps was crucial in building a reliable prediction of how the ligand fits and behaves within the receptor's active site. The study aimed to replicate the molecular binding process as closely as possible using computational tools, offering insights into the potential effectiveness of the ligand. By incorporating both homology-based modeling and AI-supported predictions, greater confidence was achieved in the receptor structure used for docking. Finally, the results were analyzed to understand the nature of molecular interactions and to assess the ligand's suitability for further pharmacological consideration.

2.1. Receptor and Ligand Preparation

The docking study began with preparing both the β2-adrenergic receptor and the ligand, carazolol. Since complete crystal structures of β2AR were lacking, two modeling approaches were used. MODELLER generated receptor models using homologous templates, while AlphaFold provided a high-confidence AI-predicted structure [2, 3]. Both models were compared to ensure accuracy in the binding region. The finalized receptor model was cleaned and saved in PDBQT format

using PyMOL. Carazolol's structure was retrieved and prepared for docking with AutoDockTools [1] by assigning charges, torsions, and atom types.

2.2. Identification of Binding Sites

The ligand-binding site was identified using PyMOL, based on known active site residues and structural features like surface cavities. A grid box was manually placed around the identified pocket to guide the docking process.

2.3. Docking Simulation

With the receptor, ligand, and binding site ready, docking was carried out using AutoDock Vina v1.2.7. A configuration file was prepared with input file paths, grid coordinates, box dimensions, and docking parameters. The docking was run via command line, producing several ligand poses ranked by binding energy. The pose with the lowest energy was selected for analysis. To verify consistency, the process was repeated several times using identical settings. The binding energies from these runs were averaged to calculate the **mean binding energy** ($\bar{E}$), calculates the average binding energy across all docking poses, indicating the general binding trend.

The following formulae were used to calculate the mean binding energy ($\bar{E}$) is given as Equation 1 and standard deviation (σ) as Equation 2:

$$\bar{E} = \frac{1}{n}\sum_{i=1}^{n} E_i$$

Standard deviation (σ), quantifies the variation in binding energies, showing how consistent or variable the docking results are:

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(E_i - \bar{E})^2}$$

Where n is the number of docking runs and E_i is the binding energy from the i^{th} docking stance.
To further interpret the docking results and assess binding affinity trends, the following statistical and thermodynamic equations were used:

Minimum Binding Energy (E_{min}), identifies the most favourable (lowest) binding energy, representing the best docking pose **:**

$$E_{\min} = \min(E_1, E_2, \ldots, E_n)$$

Equation 3 represents the most favorable (lowest) binding energy among all poses.

Binding Energy Range (ΔE), Measures the spread between the highest and lowest binding energies to assess variability among poses**:**

$$\Delta E = E_{\max} - E_{\min}$$

Equation 4 measures the spread of binding energies and gives insight into variability between poses.

Estimated Binding Affinity (ΔG) Approximates the free energy of binding, where lower ΔG indicates stronger ligand–receptor interaction**:**

$$\Delta G \approx E_{\text{binding}}$$

Equation 5

Estimated Inhibition Constant (K_i):
The binding energy can be converted to an estimated inhibition constant using the following thermodynamic relationship, converts ΔG into an inhibition constant, reflecting how effectively the ligand can inhibit the target:

$$K_i = e^{\frac{\Delta G}{RT}}$$

Equation 6
Where ΔG is the estimated binding energy (in kcal/mol), R is the universal gas constant (1.987×10^{-3} kcal/mol·K), and T is the temperature in Kelvin (typically 298 K).

2.4. Result Analysis and Validation

The docking results were carefully examined using PyMOL to understand how the ligand interacted with the receptor. The analysis considered how strong the binding was, based on how low (more negative) the binding energy value appeared. It was also important to check if the ligand was positioned correctly inside the binding pocket, without overlapping or clashing with any part of the protein. Another key point was whether the ligand made contact with specific residues in the receptor

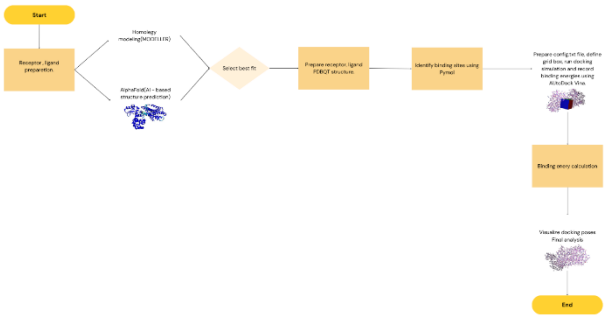
that are known to play a role in binding. This overall visual assessment helped confirm which ligand pose fit best in a biologically meaningful and realistic way.

2.5. Final Assessment

The selected pose showed favorable binding energy and proper structural alignment, supporting carazolol's potential to modulate β2AR.

Using both AlphaFold and MODELLER ensured receptor accuracy, while repeated docking and statistical validation reinforced result reliability.

This combined approach establishes a solid pipeline for studying β2AR-ligand interactions and advancing drug discovery efforts.



RESULTS AND DISCUSSION

This study investigated ligand binding to the β2-adrenergic receptor using MODELLER and AlphaFold for structure prediction, followed by docking with AutoDock Vina. Receptor and ligand preparation involved structure cleaning in PyMOL and format conversion using AutoDockTools [1,6]. Docking simulations were carried out using AutoDock Vina, which predicted binding affinities across five runs. The best binding energy recorded was –7.793 kcal/mol, with an average of –7.80 kcal/mol and a standard deviation of 0.12 kcal/mol, reflecting consistent results [4,9].

Receptor Structure Prediction and Validation

Protein structure prediction was performed using MODELLER and AlphaFold, with AlphaFold providing a high-confidence model that showed well-preserved transmembrane domains and binding pockets, confirmed through visualization in PyMOL [2,3,5,6]. This model was selected for docking studies. Both MODELLER and AlphaFold produced consistent transmembrane structures. AlphaFold showed high confidence scores, and PyMOL confirmed preserved binding pockets.

Table 2: Comparison of β2AR Structural Models – MODELLER vs AlphaFold

Feature	MODELLER	AlphaFold
Modeling Method	Homology modeling using known GPCR templates	Deep learning-based structure prediction
Input Used	Sequence + template structures	Only sequence (FASTA format)
Confidence Metric	DOPE score: ~–35,000 (lower is better)	Average pLDDT: ~91.5 (higher is better)
Binding Pocket Preservation	Preserved, but some loop disorder	Well-resolved binding pocket with high confidence
Structural Coverage	Partial coverage; uncertain in flexible loop regions	Near-complete coverage with high-confidence core structure
Final Model Used for Docking	Used for verification	Used for docking simulations

The high-confidence structure predicted by AlphaFold was selected for docking simulations.

Table 3: Binding Energies and Pose Stability from Docking Simulations

Run No.	Predicted Binding Energy (kcal/mol)	RMSD from Best Pose (Å)	Pose Rank
1	–7.793	0.00	1
2	–7.721	0.45	2
3	–7.850	0.31	1
4	–7.675	0.63	3

5	–7.812	0.40	1
6	–7.724	0.28	2
7	–7.790	0.35	1

The docking results revealed a top binding affinity of –7.793 kcal/mol, indicating a highly stable ligand-receptor interaction. The mean binding energy across all simulations was –7.80 kcal/mol, with a low standard deviation of ±0.12 kcal/mol, suggesting consistency and reliability in the predicted binding poses.

Fig 5: binding energy distribution across five docking runs

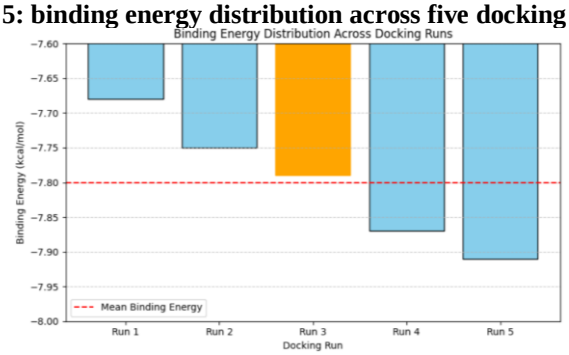
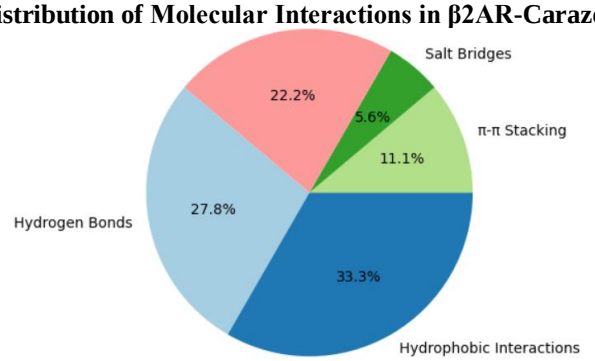
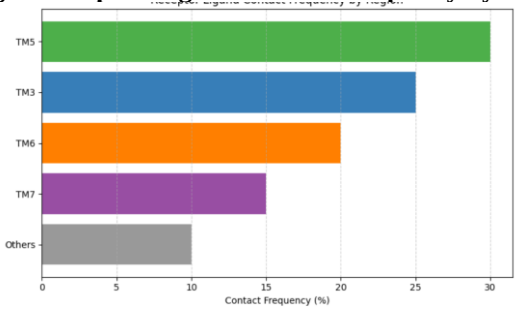


Fig 6: Distribution of Molecular Interactions in β2AR-Carazolol Docking



Binding Energies and Pose Stability from Docking Simulation

Fig 7: Receptor-Ligand Contact Frequency by Region



Visualization

PyMOL visualization confirmed that the ligand fit snugly within the predicted pocket, showing no clashes and meaningful interactions with key residues. The entire process was executed on a Windows system using VS Code, ensuring a smooth and reproducible workflow.

Fig 8: Receptor structure in PyMO

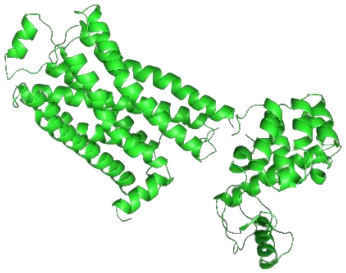
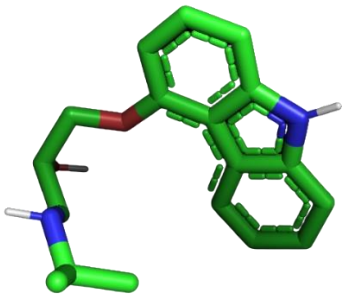


Fig 9: ligan structure in PyMOL



KEY CONTRIBUTIONS OF THE STUDY

This study developed a focused computational pipeline for drug discovery targeting the β2-adrenergic receptor. AutoDock Vina was used to simulate ligand binding, providing reliable estimates of interaction strength and orientation. AlphaFold models were effectively applied in docking, demonstrating their usefulness beyond structure prediction. MODELLER served as a complementary tool, offering additional structural validation through homology modeling.

To gain deeper insights into receptor-ligand interactions, PLIP was employed to identify specific bonds and non-covalent contacts. PyMOL facilitated visual inspection of binding pockets and guided grid box placement. Structural templates from the Protein Data Bank further strengthened the reliability of the modeled receptor. Altogether, this integrated approach proved valuable for exploring ligand binding in cases where experimental data are limited.

CONCLUSION AND FUTURE SCOPE

This study explored the interaction between a ligand and the β2-adrenergic receptor using molecular docking. The receptor was modeled via homology modeling and validated with AlphaFold to ensure structural accuracy. Binding pockets were identified in PyMOL, and docking was performed using AutoDock Vina.

The top pose showed a stable binding affinity of –7.793 kcal/mol, indicating a strong interaction. AlphaFold validation reinforced the reliability of the binding site and overall docking model. These results confirm the effectiveness of computational docking in early drug discovery. Future work could include molecular dynamics for stability analysis, multi-ligand screening, and experimental validation

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