Using LeDock as a docking tool for computational drug design

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Abstract. Computer-aided drug design (CADD) is an emerging tool for research and drug development process as it reduces the time taken for the process of drug development and expense. Molecular docking technology, as one of the main methods, has been widely used in many fields of drug development. Based on the dopamine D₃ receptor target, this paper describes the method of molecular docking using LeDock software (Windows version) in combination with the docking process of eticlopride ligand and D₃ receptor. This method can predict the binding mode of ligands to proteins, including binding energy, binding sites and attractive interactions types. Four representative D₃ receptor ligands, including BP897, NGB2904, FAUC365 and SB277011A, were respectively docked with D₃ receptor by this method. By analyzing the docking results, we can conclude that the molecular docking method using LeDock software plays an important role in the drug design process.

1. Introduction

New drug research and development is a complex engineering of high investment, high risk and long cycle[1]. Scientists have been working to develop new technologies to increase the success rate of new drug development and reduce expenses. Hence CADD has emerged as a powerful technique and promising strategy in the development of new drug[2].

Computer simulation was utilized in this method to calculate the relationship between drugs and receptor biomacromolecules. Structure-based drug design[3] (SBDD) and ligand-based drug design[4] (LBDD) are the two general types of CADD approaches in existence. Molecular docking[5] has developed into the most widely used and most successful method in SBDD since the 1960s. Molecular docking is a drug action found in the Lock and Key model[6], which is between the receptor and ligand. Molecular docking aims to predict the experimental binding modes and affinities of small molecules within the binding site of particular receptor targets[7]. LeDock is designed for fast and accurate flexible docking of small molecules into a protein. The graphic version on Windows operating system greatly simplifies common multiple sophisticated docking procedures for medicinal chemists.

Dopamine receptors belong to a super-family of G-protein coupled receptors (GPCRs). Five dopaminergic receptors have been cloned, characterized and classified in two families, the D₁-like family (D₁- and D₅-receptor subtypes) and the D₂-like family (D₂-, D₃- and D₄-receptor subtypes) [8]. It is hypothesized that D₃ receptors (D₃R) may be involved in the pathogenesis of neurological or psychiatric diseases such as Parkinson's disease, schizophrenia and drug addiction[9].

In this paper, the method of molecular docking using LeDock software was described in combination with the docking process of eticlopride and dopamine D₃R. Several representative D₃R ligands were selected and docked by this method. Analysis of the docking results found that this
method can play an important role in the drug design process.

2. Molecular docking method
To validate the suitability of LeDock software for D₃R targets, we established a method to dock eticlopride ligand with D₃R. Molecular docking using LeDock software is generally divided into four steps: preparation of protein, preparation of ligand, docking and analysis of results.

Chien et al. [10] prepared the structure of the human dopamine D₃R in complex with eticlopride. Download the three-dimensional crystal structure of the D₃R (PDB ID: 3PBL) from the RCSB Protein Data Bank.

2.1 Protein preparation for LeDock
The download protein file 3PBL was pretreated by using UCSF Chimera (Chimera) for removal of molecules unrelated to the docking process, including hydrogen bonds, coenzymes, surfactants, etc.; deletion of ligands unrelated to binding, and then obtaining a protein crystal complex containing eticlopride ligand (3PBL1.pdb) for determining docking parameters. Deletion of eticlopride ligand to obtain blank protein for docking. The blank protein was hydrogenated and charged, and saved as 3PBL0.pdb.

2.2 Ligand Preparation
Structure of eticlopride was sketched in ChemDraw and converted into three-dimensional (3D) structure in Chem3D where the 3D structure of eticlopride was minimized energy and saved as eticlopride.mol2. All hydrogen in the ligand was removed and the ligand was hydrogenated and charged in Chimera software and saved as eticlopride0.mol2.

2.3 Docking
The geometry center of the eticlopride ligand in the crystal structure was set as the center of the enclosing box (80Å × 80Å × 80 Å) using the GetBox plugin in PyMOL.

Molecular docking of processed protein files and ligand files was performed by LeDock. The protein file to be docked (3PBL0.pdb) was selected in protein input of LePro, and the hydrogens were added automatically by running LePro. The generated box size from running Lepro was changed into 80Å × 80Å × 80 Å. And the ligand file to be docked (eticlopride0.mol2) was selected in ligand input of LeDock, and then start docking.

The docking results showed 13 different conformations, ranked according to the values of predicted binding energies (score), and the binding energy of the highest scored molecule was -7.04 kcal/mol. All the conformations appearing in the docking result were compared with 3PBL1.pdb one by one using PyMOL software. It was found that the most consistent one after docking was the first constellation, which was saved as eticlopride0.dok_0001 (PDB format).

2.4 Result Analysis
The results were analyzed using the Discovery Studio 2016 Client software in this method. The ligand eticlopride0.dok_0001 was docked into the blank protein 3PBL0.pdb in the Discovery Studio 2016 Client software. The software can be used to analyze the interaction sites between the protein and the ligand after virtual docking, and present the properties of the receptor surface, such as aromatic, hydrogen bond, charge, hydrophobicity, etc., showing the two-dimensional (2D) interactions between the protein and the ligand.
The binding sites and the forces distance between the eticlopride and the surrounding D3R residues can be seen in Fig. 1b. As shown in the diagram of the combination of eticlopride and D3R (Fig. 1c), the ethyl-pyrrolidine ring of eticlopride forms a salt bridge to Asp110. The substituted aromatic ring of eticlopride connected to the pyrrolidine by an amide bond that fits tightly within a hydrophobic cavity formed by Phe345, Ile83, Val111, Ser196, Phe346, Ser192, Val350, Ser193, Val189. The phenyl ring forms the Pi-Pi T-Shaped interaction to Phe346, and the Pi-Alkyl interaction to Val111. The CH3 and Cl on the phenyl ring form the Alkyl interaction with the surrounding D3R residues.

The result of the docking is consistent with the interactions between eticlopride and D3R residues in the reference, which proves that the docking method and the software used are applicable to the D3R.

3. Molecular docking of representative ligand
The main pharmacophore features of the ligand for the D3R are represented by: a) aromatic region (preferred by a large volume group with strong hydrophobic action); b) amide region attached to the aromatic ring; c) a spacer (usually carbon chains) with different geometric distances and spatial conformations; d) phenylpiperazine structure capable of increasing ligand affinity and selectivity (Fig. 2).

Figure 2. The pharmacophore features of the ligand for the D3R.
We selected four representative D₃R ligands (Table 1) that have been reported, and used LeDock to dock these D₃R ligands and analyze the results. These ligands were pretreated according to the ligand preparation. Molecular docking of the processed ligands and protein using LeDock software and the docking binding energy is shown in Table 1. The most consistent conformation in the docking results was chosen to analyze the interaction of the ligand with the protein.

### Table 1. Representative ligands and their docking binding energy.

| Ligand       | Chemical structure | Binding energy (kcal/mol) |
|--------------|--------------------|--------------------------|
| BP897[11]    | ![Chemical Structure](BP897.png) | -7.27                    |
| NGB2904[12]  | ![Chemical Structure](NGB2904.png) | -7.01                    |
| FAUC365[13]  | ![Chemical Structure](FAUC365.png) | -7.63                    |
| SB277011A[14]| ![Chemical Structure](SB277011A.png) | -7.58                    |
Figure 3. Interactions diagram between (a) BP897, (b) NGB2904, (c) FAUC365, (d) SB277011A and D3R.

As shown in the 2D diagram of the combination of BP897 and D3R (Fig. 4a), the naphthalene ring of BP897 is in a hydrophobic cavity formed by Val107, Phe345, Ile183, Val189, Val350, Ser193, Phe346, Ser192, Ser196, Val111, and forms the Pi-Alkyl interaction with Val111, Val189, His349 and Ile183. The N on the amide bond and the piperazine respectively form hydrogen bonds with Asp110 and Cys193.

The piperazine moiety of NGB2904 forms a salt bridge with ASP110 and the Pi-Sigma interaction with Phe345. The anthracene ring of NGB2904 forms the Pi-Anion interaction with Glu90. In addition, NGB2904 forms the Pi-Alkyl interaction with Val111, Leu89, and Val86 and forms the Alkyl interaction with Val189, Phe346, Ile183, His349, and Phe345 (Fig. 4b).

The piperazine moiety of FAUC365 forms the charge attraction with Asp110, and the amide bond forms a hydrogen bond with ASP110. The benzo-thiophene ring forms the Pi-Sulfur interaction with Phe345 and forms the Pi-Pi T-Shaped interaction with His349, forming the Pi-Alkyl interaction with Val111, Ile183, and Val189. The benzene ring moiety attached to piperazine also form the Pi-Alkyl interaction with Tyr365, Leu89 (Fig. 4c).

Hydrogen bond was formed between amide moiety of SB277011A and Asp110. A Pi-Pi T-Shaped interaction is formed between the isoquinoline ring and Phe345, and this interaction is also formed between the benzene ring moiety attached to the cyano group and Tyr365. SB277011A forms the Pi-Alkyl interaction with Ile183, Val111, Cys114, Leu89. Additionally, SB277011A forms hydrocarbon bond with the surrounding amino acid residues (Fig. 4d).

We can see from the docking results that molecular docking can predict the binding energy, binding site, binding conformation and force of ligand to target using LeDock, which is a convenient method in drug design process.

4. Conclusion
The method of molecular docking using LeDock software was described based on the dopamine D3R in this paper. It is roughly divided into four parts: protein preparation, ligand preparation, docking and analysis of results. Molecular docking of four representative D3R ligands were carried out by this method. The results predict that these D3R ligands have higher docking binding energy and can bind strongly to the receptor at the binding pocket which is consistent with the literature reports. This method is not only suitable for dopamine D3R targets, but also can be applied to various SBDD processes. Molecular docking using LeDock is an intuitive and simple way that can assist researchers in drug design, effectively reducing the cost of drug development and shortening the drug development cycle, which is of great significance in the drug development process.
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