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Comparison of Performance and Results of Autodock4 and Vina Virtual Screening Software

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***Abstract*— In the present work, molecular docking of a data set of decoys and ligands against an acetylcholinesterase protein PDB ID (5HFA) was studied, using Autodock4 and Vina software. A minimal difference was found between the results of decoys and active molecules with both programs, indicating that positive and false positive results are difficult to distinguish. The effect of the initial seed and exhaustivity was evaluated, showing that the initial settings can be manipulated and affect the results. Docking tests are cost-effective and widely used. In this work, deficiencies inherent to biological work are exposed in which the software could be improved for best performance.**

Keywords— Docking, virtual screening, Autodock

I. Introduction

Virtual screening (VS) is a tool that allows filtering of groups of substances to select those that have the best characteristics for the function of interest [1]. Docking can be used as a virtual screening tool, considering that compounds with higher affinity energy will present higher bioactivity (*fig.1*) [2]. Predictions based on simulations are the result of applying physical formulas to predict the behavior of the protein and the ligand, the set of formulas is called the force field [3]. The simulations have shown good predictive power and are widely used in the pharmaceutical industry for the design of new molecules with therapeutic potential [4].

VS technique has been used to scan the FDA database containing already approved drugs that can be repositioned or used for other diseases, drug repositioning has been used before, the clear example of this is sildenafil that had originally been it was proposed to treat pulmonary hypertension and reused for erectile dysfunction [5]. During the recent pandemic, some virtual screening studies have been conducted to find a drug in the FDA database to repurpose an approved drug that can serve as an antiviral against the virus and its main spike protein. A study found a set of 20 promising molecules including, among others, a broad spectrum antiviral (ribavirin), an anti-hepatitis B drug (telbivudine), and some vitamins, all of them safe and whose effects are already known [6]

An advantage of VS is that the molecules are already approved, and their side effects are known, so the approval process for reuse is faster. They are safe substances with low or no toxicity, the risk compared to the reward is greater. On the other hand, the development of new molecules is expensive and time consuming, something that only large pharmaceutical companies can afford. It is estimated that the pharmaceutical industry takes between 12 to 15 years to develop a new drug, considering from its design to its commercialization to the public, all this has an approximate cost of 1,200 million dollars [5], [7].

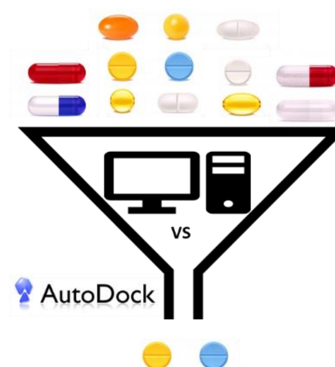

Figure 1. Virtual screening schematic representation

II. Theoretical background

Autodock4 and Autodock Vina are two widely used open-access tools for conducting interaction tests, each having been cited more than 6000 times in the last ten years. *In-silico* studies are mainly to generate hypotheses and conduct directed and better focused experimental studies or to verify and explain experimental findings [8]. The difference

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between Autodock4 and Vina software is the scoring function, in Autodock4 it is based on a semi-empirical calculation that involves the Coulomb potential, Lennard-Jones potential, desolvation volume, and the number of rotatable bonds [9]. On the other hand, the VINA score function is empirical, it uses Gaussian steric interactions, hydrogen bonds, rotatable bonds, repulsion, and torsion forces [10].

Its first version of Autodock was released in 1990, currently Autodock4 (2009) is a tool that uses a grid-based method to allow rapid evaluation of binding energy of trial conformations of ligands against a target protein. The target protein is embedded in a grid, a probe atom is then sequentially placed at each grid point and the interaction energy between the probe and the target protein is computed, the value is stored on the grid. This grid is then used as a lookup table during docking simulation [9].

Autodock4 uses a Lamarckian genetic algorithm, a population of trial conformations of the ligand is created and competes in a biological evolution manner, ultimately selecting the best binding energy individuals (negative affinity energy). Also, the software uses a semiempirical free energy force field to predict binding energies, based on a thermodynamic model with intramolecular energies in bound and unbound states and a charge-based desolvation method calibrated with a set of 188 protein-ligand experimentally well-known complexes with errors of 2-3 kcal/mol in prediction. Other considerations can be considered like receptor flexibility and covalent docking.

Later, AutoDock Vina was released in 2010 with improvements in accuracy and speed-up in calculations. The new method approach is more of "machine learning" than physics-based nature, justifying performance on test problems more than theoretical considerations following some strong approximating assumptions as described by its creators [10].

III. METODOLOGY

A. Choose of ligands

For this work, two databases were used: First, a set of molecules, 644 active compounds and 664 decoys were chosen from the DUD-e database [11], which are already experimentally tested compounds for the bioactivity of acetylcholinesterase. The second group was 67 AChE inhibitors and 67 substrates, these were obtained from CHEMBL data base [12]. Compounds were converted from SDF to PDBQT format using OpenBabel software with which energy minimization of the molecules was also performed [13].

B. Preparation of the protein structure

The enzyme acetylcholinestase was used since its structure and operation are well known. The structure of the 5HFA was chosen and downloaded from the PDB database. To perform a virtual screening based on blind coupling, the water molecules were removed from the crystallographic structure of the discharged protein, as well as the ligands present, this was done using the USFC-Chimera software, the file was saved in PDB format. Subsequently, the structure was processed using AutoDock Tools, the polar hydrogens were added, the Kollman charges were fused and finally the non-polar hydrogens. The structure was saved in PDBQT format.

C. Docking process

Autodock Vina and Autodock4 were used to perform virtual screening of ligands against the 5HFA protein. A script was created to automate the process using bash scripting, only one conformation was chosen, the best energy result in kcal/mol units with exhaustiveness of 8, the sampling box was set to cover all the protein since docking was performed blind. In the case of Autodock4, the same files were used, however the docking script configuration was done through the Raccoon software support. Additional tests were performed using Autodock VINA to analyze different configurations available in the software. Two types of tests were carried out, based on the type of starting seed, fine seed (27527408) and random seed. Tests were also made to evaluate the exhaustivity, that is, the number of evaluations that are carried out in the conformational search, 8, 16 and 32 of exhaustivity were used. The calculations were performed on a desktop PC with a 4-core i5 processor and 12 GB of RAM running Ubuntu 20.04 LTS operating system.

IV. RESULTS

The predictions show that the affinity energy calculated with Autodock4 is three units greater than the energy calculated with Vina (*fig. 2*). This means a limitation to do comparisons between the predictions of different software's. In both cases, there was no difference between the prediction of active substances and the negative controls (table II). So, in both software's it is not easy to make a distinction between an

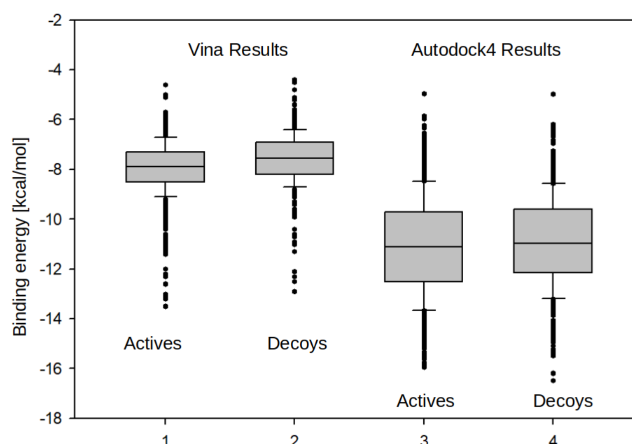

Fig. 2.-Binding energy calculated with Autodock4 and Autodock Vina using a database of active and decoy compounds against the acetylcholinesterase enzyme. N = 664, a single test of each compound with exhaustiveness of 8 in Vina and 2,500,000 in Auto

TABLE II

Type of compound	*AutoDock Vina (kcal/mol)*	*AutoDock4 (kcal/mol)*
Decoy	*-7.58 ± 1.01*	*-10.87 ± 1.83*
Active	*-7.96 ± 1.15*	*-11.10 ± 2.01*

Table 1.-Results of affinity energy obtained with AutoDock Vina and AutoDock4 using the same data set of 664 active and 664 decoy compounds against Acetylcholinesterase enzyme PDB ID (5HFA).

TABLE III

Measure of Affinity	*AutoDock Vina*		*Auto Dock4*	
	Active	Decoy	Active	Decoy
Mean*	-7.968	-7.587	-11.108	-10.878
Std. Deviation	1.154	1.019	2.015	1.835
Skewness	-1.210	-0-854	0.135	0.028
Std. Error of skewness	0.095	0.095	0.095	0.095
Minimum*	-13.500	-12.900	-15.933	-16.486
Maximum*	-4.600	-4.400	-4.954	-4.972

**Units of affinity kcal/mol*

Table 2.-Among the descriptive data, the difference between the skewness of the results. The skewness is a measure of the normality of the measurements, it can be observed that the Autodock4 results have a normal distribution (asymmetry 0.5 to 0.5), while the Vina results have a moderate obliquity in the decoys (<-0.5) and are large on active compounds (<-1).

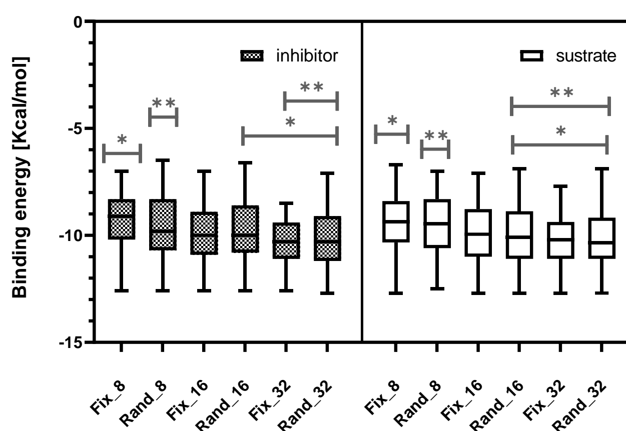

*Fig. 3. Binding energy calculated with Autodock Vina using a database of kwon substrates (n =67) and inhibitors (n = 66) against the acetylcholinesterase enzyme. A single test of each compound different exhaustiveness (8, 16, 32) and seed was fixed (Fix) (seed = 27527408) or random (Rand). * means statistical difference in each group, statistical probe ANOVA one way, Student Newman Keuls post hoc.*

active and a non-active substance only considering the result of the affinity energy. In both cases, the decoys had higher affinity energy (considering that they are negative values), there is a difference between both groups, although it is small, and it is not possible to distinguish between active and non-active substances. A higher standard deviation can also be observed in the results obtained from Autodock4, which represents a greater range of variability between the predictions (table III).

The results of the variations in the Autodock VINA settings showed a significant change between the groups. There is a statistical difference between the results of tests with inhibitors with fixed seed and an exhaustivity of 8, with respect to the completeness in random seed at 16 exhaustivity and 32, with both seed configurations. The same occurs with the exhaustivity of 8 and the random seed, and in 32 with both seed configurations. In the case of substrates, the exhaustivity of 8 with respect to that of 32 is where the greatest statistical differences can be observed ($p < 0.05$). When all results are averaged only to compare inhibitors versus substrates, no significant differences are observed, there is no way to distinguish AChE agonist substrates from antagonist inhibitors.

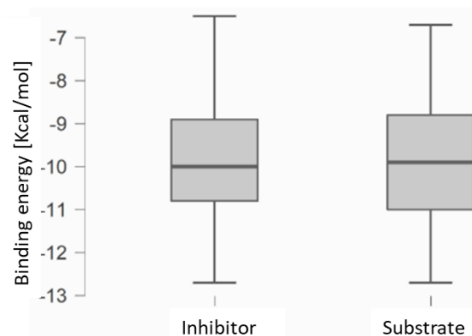

Fig. 4. Binding energy between acetylcholinesterase enzyme and known ligands, calculated with Autodock Vina and Autodock4 using a database of kwon substrates (n =67) and inhibitors (n = 66). Blind docking with exaustity of 8 and random seed.

DISCUSSION

Decoys represent a problem for computational prediction studies based on coupling assays, since they represent false positives that show high affinity energy and in experimental tests, they do not have good activity. False positives represent economic and time wasters [14]. In this study, it is shown that the active compounds and decoys cannot be distinguished with Autodock4 or Autodock Vina, although a slight decrease in the affinity energy was found. One of the relevant differences in the results is that the Autodock4 predictions have lower affinity energy, but the proportionality between compounds is maintained using the different computer programs. The results obtained must not be comparable between different software, they must have points of comparison calculated with the same software.

On the other hand, it was shown that the range of results obtained with Autodock4 is wider than those obtained with Vina. Having a wider range makes it possible to distinguish the best ligands from the rest of the compounds with greater security. Also, the normal distribution measured by the degree of skewness shows that Autodock4 software has the advantage of separating compounds into larger intervals and distinguishing substances. Autodock4 has computational advantages over Vina, although Vina is faster.

The results of the configuration variations showed that they can alter the values found, by altering the seed and the exhaustivity, the virtual screening results can be manipulated, increasing the risk of false positives and negatives. The most common configuration is to have a random seed and an exhaustiveness of 8. However, as found in this study, there is no way to distinguish between active compounds and decoys, and between active compounds there is no way to distinguish between substrates (agonists) and inhibitors (antagonists). It

is necessary to carry out a more in-depth analysis or implement new algorithms that make it possible to distinguish between the type of activity of the ligands. This study exposes some of the limitations on molecular docking software as a virtual screening tool. New approaches that use machine learning could improve these deficiencies [14].

CONCLUSION

Autodock4 software makes predictions with lower affinity energy than Autodock Vina. The distribution of the results in a wider interval and with a normal distribution in Autodock4 with respect to Autodock VINA. Based on the resulting affinity energy alone, there is no way to distinguish between active compounds and decoys, and agonist compounds cannot be distinguished from antagonists. Affinity energy is a measure that should be taken with caution in its interpretation [15].

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