HTP SurflexDock: a web tool for Structure-Based Virtual Screening analysis based on the Ensemble Docking protocol

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Abstract. *Structure-Based Virtual Screening (SVBS) is a technique traditionally used to find a set of specific inhibitors for a receptor structure during the preliminary stages of drug discovery studies. However, more than 90% of SBVS best ranks compounds do not have the expected biological effect at the end of the process. In this context, strategies to increase the success rate must be employed to ensure the experiment's success. Here, we introduce the HTP SurflexDock, a tool that improves the success rate of SBVS experiments through two strategies: First, the ensemble docking protocol enables the simulation of the implicit flexibility of the receptor structure. Second, a post-processing steps allows the user to rescore promising compounds by expanding the conformational space search or estimating the binding free energy through the MM/PBSA protocol. HTP SurflexDock is useful when dealing with flexible receptors and when structural information about the receptor contact area is insufficient or optimized for a specific ligand type. HTP SurflexDock is freely available as a web service or may be downloaded at https://htpsurflexdock.biocomp.uenf.br/.*

1. Introduction

Structure-based virtual screening (SVBS) is a technique widely used in early-stage drug discovery that uses molecular docking and a virtual dataset of compounds to explore the 3D contact surface of a drug target and classify the compounds using a scoring function. However, less than 10% of the best hits found in SBVS have any activity in *in vitro* experiments at acceptable concentrations [Perola 2006]. In this way, several strategies are employed to optimize the investigation and improve its success rate. These include pre-processing steps, such as adding receptor's flexibility, and post-processing steps to filter out the hits with better chances of success in the subsequent stages of drug discovery [Antunes et al. 2015].

Protein flexibility is critical for various biological processes such as signal transduction, enzymatic functions, and substrate recognition [Wang et al. 2020]. A protein can undergo significant conformational changes upon interaction with a substrate due to induced fit and conformational effects [Antunes et al. 2015]. However, one of the most critical challenges for SBVS is to account for the flexibility of the receptor when docking with the ligand, as the explicit techniques to account for flexibility are computationally intensive [Chaudhury and Gray 2008; Sinko et al. 2013]. In this way, rigid docking tools are typically used, not considering the effects of flexibility on protein-ligand interactions and leading to problems known as the receptor memory effect. Consequently, the receptor recognizes only some types of ligands, and other interesting compounds may not be identified [Feixas et al. 2014]. On the other hand, the generation of ensembles of receptor conformations before docking allows the experiment to simulate, on some level, the implicit flexibility of the receptor and allows better accommodate the ligands on their contact surface [Kolodzik et al. 2018]. This technique, known as ensemble docking, uses receptor conformations obtained from molecular simulations or

crystallography to make the SBVS experiment unbiased for a particular type of ligand [Lavecchia and Di Giovanni 2013].

In general, the accuracy of the scoring functions is not sufficient for the fine classification of the compounds, as the SBVS tools do not always correctly represent the complexity of the interaction of the ligand with the receptor. This may favor poses with artifacts such as steric conflicts, malformed hydrogen bonds, and other structural problems [Lionta et al. 2014]. Therefore, post-processing steps promote the exploration of the active site conformational space by generating new poses or using different protocols to estimate the binding free energy of the protein-ligand interaction. Methods such as MM/PBSA (Molecular mechanics Poisson-Boltzmann surface area) are commonly used to rescore compounds [Wang et al. 2019].

The MM/PBSA protocol estimates the relative free energy of binding using an ensemble of conformations obtained from the molecular dynamics (MD) of the protein-ligand interaction in an aqueous solvent and further calculating: (i) the potential energy change in vacuum (sum of binding energies, angular and torsional contributions, Van der Waals interactions and electrostatic energies), (ii) the desolvation energy of the components of the system and (iii) the conformational entropy of the gas-phase complex [Wang et al. 2018]. The MM/PBSA protocol is widely used for rescoring compounds in SBVS experiments because it compromises low computational cost and high accuracy [Ren et al. 2020].

In this context, we present the HTP SurflexDock, a web tool for performing SBVS experiments using the ensemble docking protocol with four conformations of the receptor obtained from 5 nanoseconds MD in explicit solvent (https://htpsurflexdock.biocomp.uenf.br/). In addition, it includes post-processing phases that increase the conformational search space and allows hit rescoring by estimating the binding free energy using the MM/PBSA protocol.

2. Platform description

A typical HTP SurflexDock experiment proceeds as follows: first, the user loads a three-dimensional structure of the receptor (format RCSB PDB) and a dataset of small molecules to be challenged against the receptor (format AutoDock PDBQT). The server then ranks compounds according to the ΔG calculated for each conformation of the ensemble and presents the results through a user-friendly interface.

Based on our previous work [De Almeida Filho and Fernandez 2019], the ensemble docking protocol builds the ensemble from the original receptor structure. Another three conformations are obtained from five nanoseconds MD using Gromacs 5.1.5 [Abraham et al. 2015]. The MD relaxes the receptor by optimizing its interaction with the solvent and allowing it to adopt other conformations. The simulation trajectory is submitted to the clusterization protocol, and conformations with a maximum binding site RMSD of 0.10 to 0.20 nm are grouped using the GROMOS algorithm [Daura et al. 1999]. Then, a representative structure of the three most representative groups is added to the receptor ensemble. The pipeline challenges the dataset of compounds against each conformation of the ensemble using AutoDock software [Norgan et al. 2011] and ADT scripts [Morris et al. 2009]. First, ten poses are calculated for each complex using 2.5x106 energy inference and the Lamarckian Genetic Algorithm (LGA) [Morris et al. 1998]. Finally, the HTP SurflexDock generates a score table containing the ΔG of the best pose of each calculated complex (Figure 1).

The HTP SurflexDock presents two options for post-processing protocols: First, it allows expanding the conformational search space of up to 10 user-defined compounds through a new cycle of docking experiment.

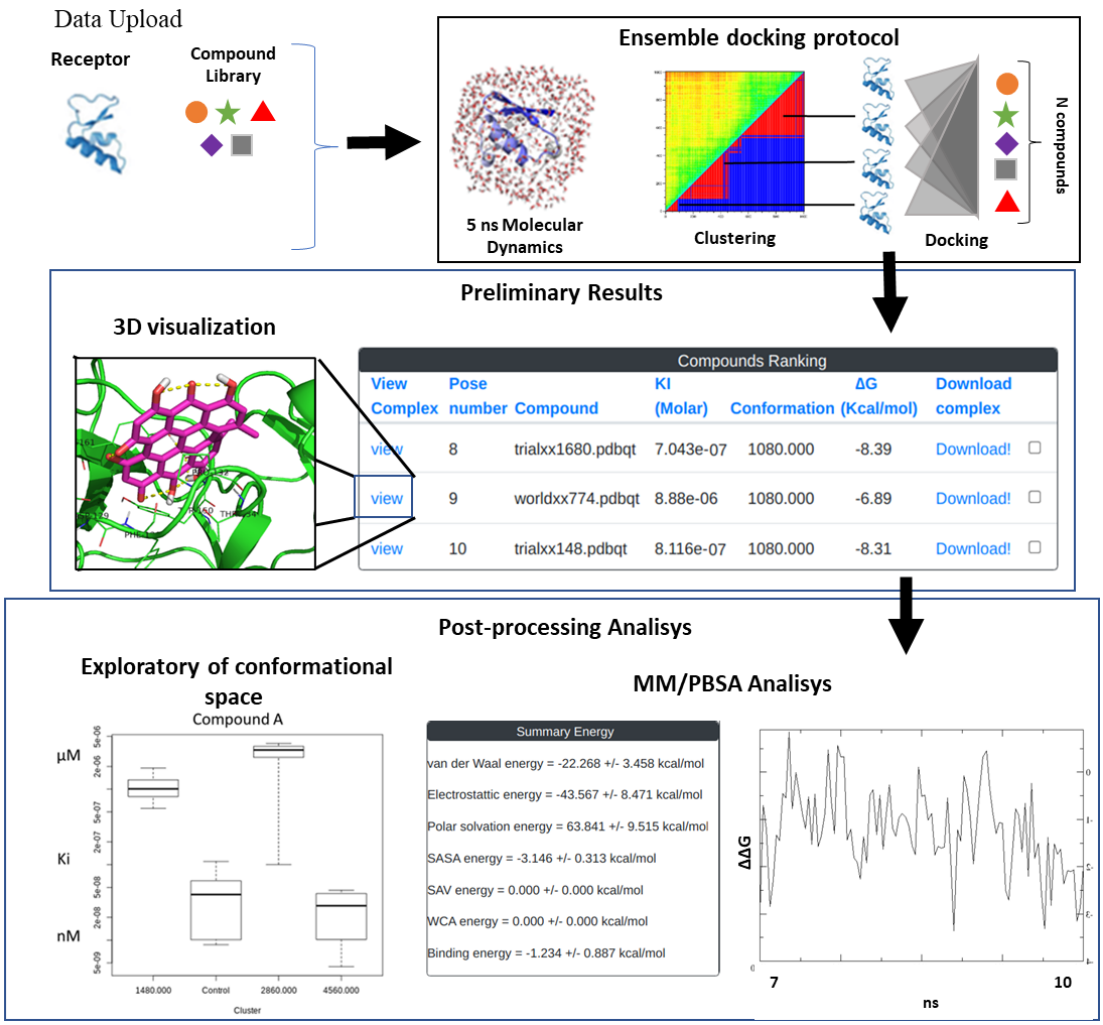

Figure 1. Workflow of HTP SurflexDock. (A) The user loads the receptor structure and a library of compounds at https://htpsurflexdock.biocomp.uenf.br/. (B) The ensemble docking pipeline is run, obtaining 3 receptor conformations from a 5-ns molecular simulation and using the original conformation as a control. The compounds are docked into the ensemble conformations. (C) The results are presented in the form of a table where the user can visualize the complex or perform post-processing tasks. (D) HTP SurflexDock allows exploratory analysis of the conformational space through boxplot diagrams and MM /PBSA calculation of the complex.

The exploratory of conformational space calculates up to 30 new poses. It allows the user to analyze the compounds qualitatively by studying the interactions calculated in the poses using AutoDock and ADT script again. Furthermore, the tool estimates the relative free energy of binding through MM/PBSA protocol implemented in the software g_mmpbsa [Ren et al. 2020]. The binding free energy inference pipeline starts in a pre-processing step, where the topology parameters from the Amber99sb force field [Song et al. 2019] for the ligand are obtained by the software ACPYPE [Bernardi et al. 2019]. Amber21 [Case et al. 2020] and Openbabel program [O'Boyle et al. 2011] are used for the addition of Gasteiger charges [Gasteiger and Marsili 1978]. Then, the Gromacs 5.1.5 software generates a ten nanosecond MD of the complex. The first seven nanoseconds are considered equilibrium phase, and the final three nanoseconds are used by g_mmpbsa to calculate the binding free energy components of the complex. In the end, the mmpbsa.py script [Ren et al. 2020] obtains the averages of the energy contributions, and a plot of the variation of ΔG against time (ΔΔG) is generated through the matplotlib library [Hunter 2007].

3. Evaluating the HTP SurflexDock's ensemble docking protocol

We evaluated HTP SurflexDock in discriminating active and inactive compounds using a case study with human angiotensin-converting enzyme I (ACE), a zinc-dependent metalloprotease that converts angiotensin I to angiotensin II, a critical vasoconstrictor [Masuyer et al. 2012]. Therefore,

this enzyme has become a classic target of several drugs for treating cardiovascular diseases and the best known of these molecules is the bradykinin potentiating peptides (BPP) analog Captopril [Wisnasari et al. 2016; Bateman et al., 2017; Evans et al., 2016]. However, this drug has had many adverse effects, so there is a general interest in finding new and safe drugs for this target (Fernandez et al., 2004).

3.1 Methodology

In this experiment, the three-dimensional structure for the C-terminal (PDB id 1O86) and N-terminal (PDB id 5amb) domains of ACE was retrieved from RSCB PDB. AutoModel modeling software [De A Filho et al., 2018] was used to complete the regions without structural information of both domains. The compound dataset was generated using the DUD/ACE (Database of Useful Decoys) [Mysinger et al. 2012], which contains active (ligands) and inactive (decoys) challenging compounds. However, eleven thiol ligands were removed from this library because they showed poor performance in preliminary experiments. Nine ligands of different chemotypes, obtained from human ACE crystallographic data in the PDB, were added to maintain the ligand/decoy ratio in the dataset. The final dataset used in the experiments consisted of 1797 decoy molecules and 44 ligands, classified as 16 carboxyl compounds, five phosphinic compounds, 17 thiol compounds, and 6 BPPs compounds. All selected molecules were converted to pdbqt format using the HTP SurflexDock conversion tool. After conversion, the molecules were renamed according to their classification with prefixes '*ligandxx*' and '*decoyxxxx*'.

The HTP SurflexDock was configured using default parameters to challenge the compounds near Glu411 for the C-terminal domain and Glu311 for the N-terminal domain. The open-source script roc2py [OEChem 2012] generated ROC graphs to evaluate the results qualitatively. This methodology consists of a two-dimensional plot where the y-axis indicates the number of ligands found (TPR - True Positive Rate) and the x-axis shows the classification's number of decoys found as active (FPR - False Positive Rate).

Because only the fraction of the first enriched molecules of an SBVS experiment would be used for future laboratory confirmations, these molecules are typically the best-scored ones [Truchon and Bayly 2007]. Taking this into account, another critical parameter evaluated in the experiments was the "early enrichment", defined as the quantification of the first enriched ligands in the experimentation.

4. Results

To ensure representative ensemble in HTP SurflexDock, structural sampling in 5ns simulations for the definition of the receptor structures were used. A significant improvement was observed in at least one of the conformations of the N-terminal domain (conformation t=4460 ps), in which about 4% of the ligands were better enriched than in the control conformation (Figure 2).

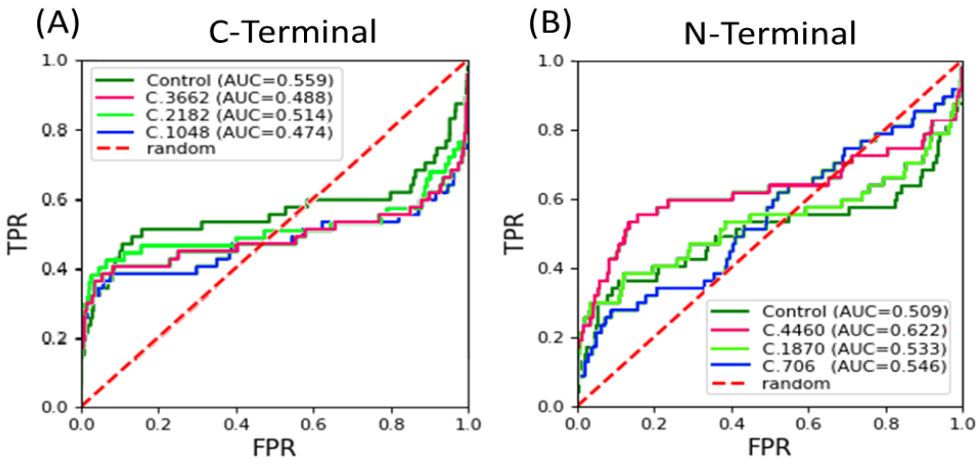

Figure 2: Early enrichment of the SBVS using receptor's conformations from 5 ns molecular simulation. (A) ACE C-terminal domain. (B) ACE N-terminal domain.

Regarding ligand "early enrichment", the experiment with the 5ns ensemble in HTP SurflexDock was able to change the enrichment profile by enriching twice as many ligands in the t=2182 ps conformation and enriching new phosphinic type ligands in the t=3662 ps conformation in the human ACE C-terminal domain (Table I). In the case of the experiment with the N-terminal domain, the enrichment profile of the t=4460 ps conformation was changed, and more BPP-type ligands were found (Table I). In this context, the expansion of the conformational space generated by ensemble docking allowed the number of different ligands enriched between the first 15 compounds to increase by 87% when using the 5ns simulation ensemble.

Finally, we analyzed different "early enrichment" extracts from both human ACE domains. The results showed that the implementation of the ensemble docking methodology allowed an increase in initial enrichment of the ligands if compared with the control experiment. Up to 47% more compounds were enriched in the first 1/5 of obtained results for the human ACE N-terminal domain (Table II).

Table I – Early enrichment of the 15 best dockings using conformations obtained from the 5ns simulation of the hACE N-Terminal and C-Terminal domain.

hACE N-Terminal domain							
Control		**Conf. t=1048**		**Conf. t=2182**		**Conf. t=3662**	
Compound	**ΔG**	**Compound**	**ΔG**	**Compound**	**ΔG**	**Compound**	**ΔG**
BPP5_3	-11,1	BPP5_3	-11,3	BPP5a	-12,1	BPP3	-11,1
RX3	-10,5	BPP3	-10,6	RX3	-11,1	ligand_15	-10,3
ligand_15	-10,5	ligand_15	-10,4	BPP3	-11,1	BPP5a	-10,2
decoys_600	-10,5	BPP5_4	-10	ligand_15	-10,7	BPP5_3	-10
BPP3	-10,5	ligand_24	-9,92	ligand_24	-10,7	decoys_554	-9,46
ligand_33	-10,5	RX3	-9,71	BPP5_3	-10,5	RX3	-9,46
BPP5_2	-10,3	ligand_31	-9,63	ligand_34	-9,91	ligand_23	-9,27
BPP5a	-10,3	decoys_53	-9,6	decoys_564	-9,61	decoys_600	-9,19
decoys_156	-10,3	decoys_1699	-9,56	ligand_42	-9,6	decoys_534	-9,1
decoys_534	-10,2	decoys_564	-9,51	decoys_156	-9,57	ligand_42	-8,95
decoys_1281	-10,2	BPP5a	-9,5	ligand_37	-9,35	BPP4	-8,93
decoys_1321	-10,2	decoys_939	-9,49	BPP5_2	-9,34	decoys_815	-8,87
decoys_1205	-10,1	BPP5_2	-9,29	decoys_1699	-9,32	decoys_522	-8,83
decoys_554	-9,92	decoys_165	-9,27	ligand_33	-9,32	decoys_1773	-8,8
hACE C-Terminal domain							
Control		**Conf. t=706**		**Conf. t=1870**		**Conf. t=4460**	
Compound	**ΔG**	**Compound**	**ΔG**	**Compound**	**ΔG**	**Compound**	**ΔG**
BPP3	-12,3	BPP3	-10,75	RX3	-11,30	BPP5_3	-11,60
ligand_15	-11,3	RX3	-10,60	ligand_15	-11,10	RX3	-11,40
RX3	-11,0	ligand_15	-10,42	BPP5_3	-10,90	BPP3	-10,50
ligand_24	-10,8	ligand_27	-10,00	decoys_338	-9,86	ligand_42	-10,40
decoys_1574	-10,7	decoys_1270	-9,98	BPP5_2	-9,85	BPP5a	-10,30
decoys_1398	-10,7	decoys_1716	-9,88	decoys_599	-9,84	BPP5_2	-10,30
BPP4	-10,6	decoys_608	-9,82	BPP3	-9,76	ligand_15	-10,20
BPP5_3	-10,6	decoys_320	-9,79	ligand_24	-9,71	ligand_23	-9,90
ligand_34	-10,5	decoys_284	-9,75	ligand_23	-9,69	decoys_1398	-9,80
decoys_1269	-10,5	decoys_1398	-9,71	decoys_1773	-9,61	decoys_1773	-9,79
decoys_1270	-10,4	decoys_913	-9,69	decoys_913	-9,53	decoys_338	-9,75
decoys_284	-10,4	decoys_286	-9,58	decoys_619	-9,50	decoys_619	-9,74
decoys_1152	-10,4	decoys_619	-9,58	decoys_1270	-9,46	decoys_310	-9,68
decoys_1320	-10,3	decoys_310	-9,53	decoys_1276	-9,45	decoys_534	-9,65
decoys_848	-10,3	decoys_613	-9,49	decoys_705	-9,42	decoys_531	-9,64

BPP Phosphinic Carboxylic

Table II: Number of ligands enriched by conformation of the ensemble obtained from the 5 ns simulation

C-Terminal Domain					
Ranked experimental subset	**0.55%:**first 10	**0.8%**:first 15	**5%**:first 92	**10%**:first 184	**20%**:first 369
Original structure (Control)	8	8	16	21*	23*
Conformation I (1048 ps)	7	9	16	18	18
Conformation II (2188 ps) X	8*	11*	18*	19	20
Conformation III (3662 ps)	7	8	16	18	18
N-Terminal Domain					
Ranked experimental subset	**0.55%**:first 10	**0.8%**:first 15	**5%**:first 92	**10%**:first 184	**20%**:first 369
Original structure (Control)	4	6	11	16	17
Conformation I (706 ps)	4	4	9	14	16
Conformation II (1870 ps)	7	7	14	14	18
Conformation III (4460 ps) X	8*	8*	14*	19*	25*

5. Conclusion

Here we introduce HTP SurflexDock, a web server that allows users to perform SBVS experiments intuitively with ensemble docking, using a few parameters defining the active site of the receptor and general experimental conditions. Since there is no pre-established protocol for ensemble docking, we evaluated the effects of the protocol in a challenging case study using the human ACE N and C-terminal domains. Our results showed that the pipeline implemented in HTP SurflexDock could avoid the receptor "memory effect" caused by the crystallographic data over the rigid docking by allowing the enrichment of chemical profiles for different ligands. In addition, ensemble docking reduced the number of false positives among the best-detected compounds, making the tool perform a more efficient SBVS experiment.

Furthermore, the HTP SurflexDock was also employed in a recent study to repurpose antiviral agents for SARS-CoV2. Here presented tool tested 3400 molecules from the ZINC15 database [Sterling and Irwin 2015] against the SARS-CoV2 main protease (Mpro) and SARS-CoV2 RNA-dependent RNA polymerase (RdRp) enzymes. A shortcut list of promising compounds was subjected to ΔΔG calculation using the MM/PBSA post-processing protocol. SBVS analysis revealed several antiretrovirals, antifungal, and antitumor agents that inhibit SARS-CoV 2 enzymes. Moreover, Hypericin obtained promising results with a $\Delta\Delta G = -22.704 \pm 4.008$ Kcal/mol, and thus its antiviral activity was evaluated in Vero E6 cells incubated with the new coronavirus. The results indicated that Hypericin caused a significant reduction of viral RNA in the supernatant at concentrations between 10 and 100 µM and exhibited a low cytotoxic activity at these concentrations [Matos et al. 2022].

These presented features certify the HTP SuflexDock as a valuable resource for SBVS experiments with flexible, poorly characterized, or predicted by homology modeling receptors and drug repurposing experiments. Users can run entire SBVS experiments in a simple web environment without worrying about maintaining a high-performance computer pointing a simple browser to https://htpsurflexdock.biocomp.uenf.br/. Nevertheless, complex experiments can be performed on local computers using the standalone version of HTP SurflexDock free available for academic users at (https://bitbucket.org/jlalmeidaf/htp_4/src/master/).

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