Ligand binding mode analysis using new 5-HT_{1A} receptor models developed by ALiBERO methodology

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Main objective

The search for the best ensemble of 5-HT_{1A}R homology models with application of ALiBERO (Automated Ligand-guided Backbone Ensemble Receptor Optimization) [1].

ALiBERO is a new computational tool which expands the pocket selection from single to multiple in an automatic way. Pocket selection procedure uses comprehensive combinatorial search enriching the final receptor ensemble by only those that maximize the discrimination of active compounds from decoys. ALiBERO framework is a heuristic search composed of two main steps: generation of multiple receptor conformations and selection of the best individual conformations according to the flexible-docking static-receptor small-scale docking performance. The best performing pockets are selected for the next generation of receptor conformers. This iterative process is repeated until the threshold for the fitness function is reached.

Optimized parameters

- different clustering approach of active compounds used as a training set (ICM clustering, MOLPRINT2D and manual clustering [2]) (Figure 2.)
- template (ß-adrenergic (2RH1) [3] and the recently published 5-HT1B (4IAR) [4] - the closest 5-HT_{1A}R homologue) (Figure 3.)
- selection method of inactives for training set composition.

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26 ALiBERO scenarios

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Evaluation

Each scenario was evaluated in the virtual screening-like experiment with 100 diverse 5-HT_{1A}R ligands and 900 DUD-like decoys. Each experiment was assessed by area under the receiver operating characteristic curve (ROC) [5], describing the ability of classification procedure to recognize true positives and negatives. Normalized Square root AUC (NSQ_AUC) metric [6] which is especially sensitive on early hit enrichment was also calculated.

Binding mode analysis

A set of 40 structurally diversed 5-HT_{1A} receptor ligands (arylpiperazines, indoles, tetralines) with putative binding mode proposed in the literature were docked to the top scored ensemble of pockets. Binding modes were analyzed by means of the SIFt methodology [7].

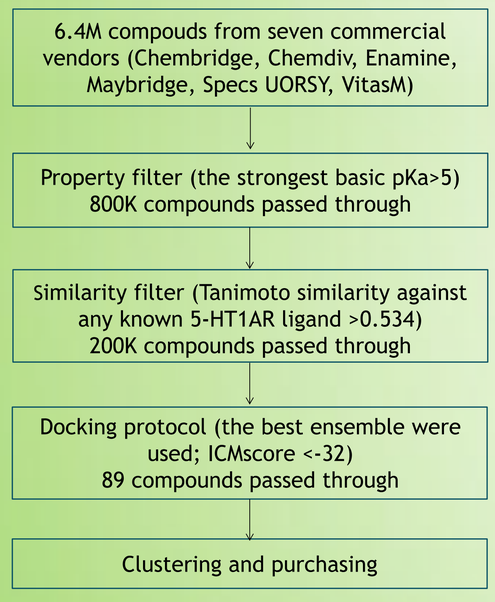
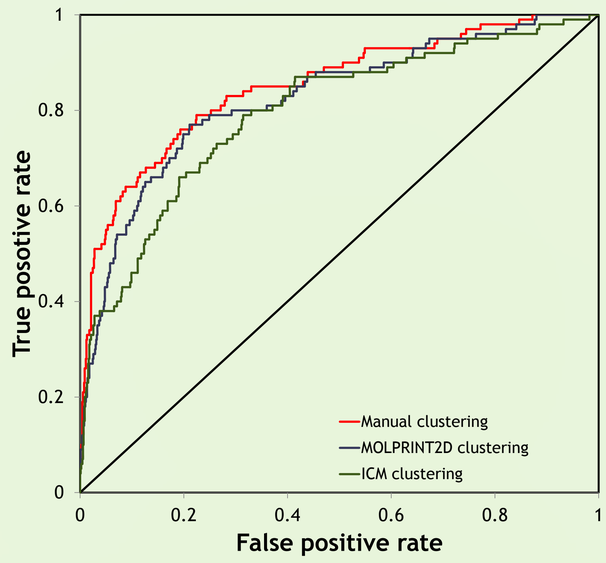


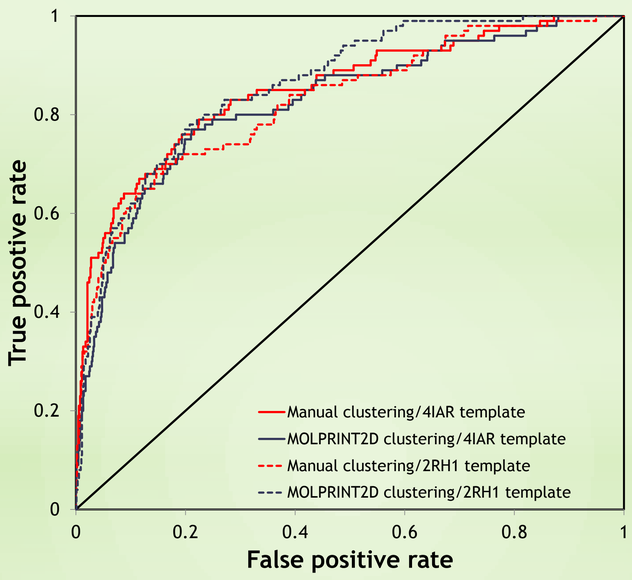
Figure 1. Virtual screening campaign workflow.



Clustering method[a]	AUC	NSQ_AUC
Manual	0.851	0.675
MOLPRINT2D	0.825	0.613
ICM clustering	0.796	0.554

[a]All ensembles were developed on 4IAR template and on training set with actives/inactives ratio 1:1

Figure 2. Evaluation of actives clustering approach impact on ALiBERO efficiency



Clustering method/template[a]	AUC	NSQ_AUC
Manual/2RH1	0.826	0.625
MOLPRINT2D/2RH1	0.861	0.666
Manual/4IAR	0.851	0.675
MOLPRINT2D/4IAR	0.825	0.613

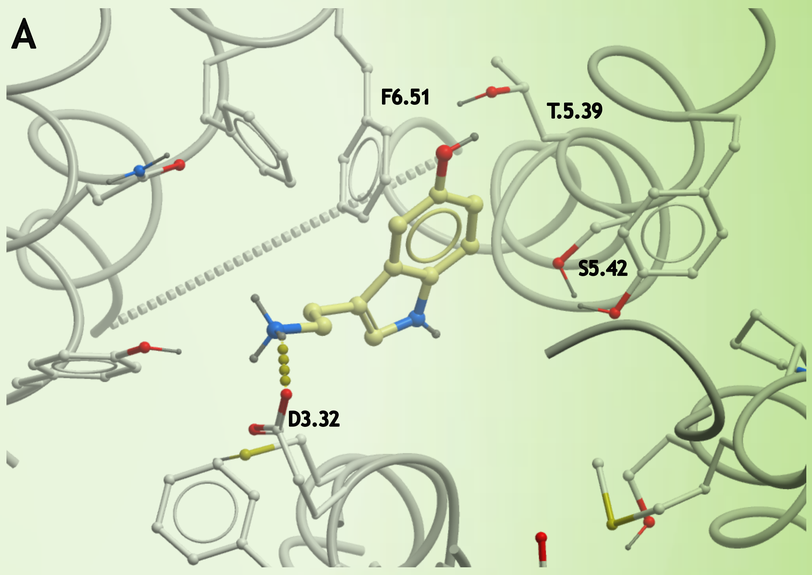
[a]All ensembles were developed on training set with actives/inactives ratio 1:1

Figure 3. Evaluation of template impact on ALiBERO efficiency

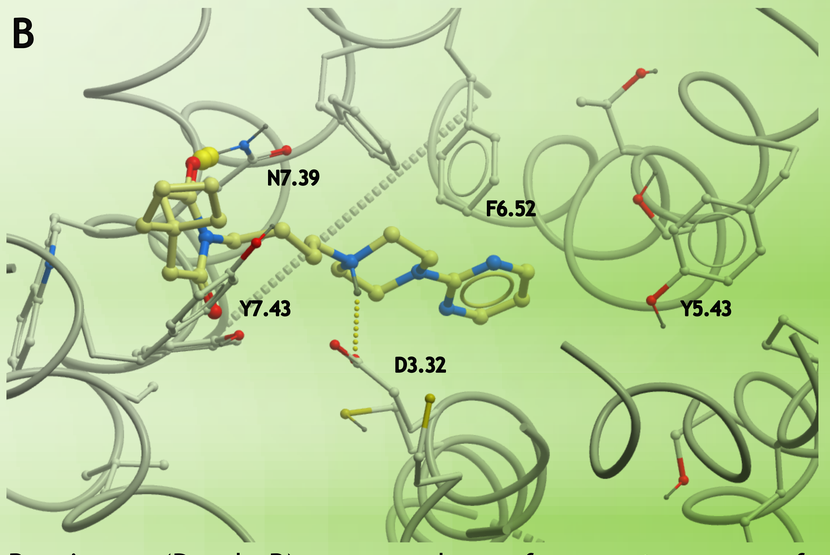
Conclusions

ALiBERO methodology is a new, robust approach for structure-based virtual screening. In this research 26 different scenarios were evaluated in order to investigate the influence various parameters on the performance of the method. As the results show, the composition of the training set (manual clustering and active to inactive ratio of 1) is of great importance. On the other hand, the template used did not affect of the algorithm in any significant manner. The best ensemble of receptors was used as a final filter in virtual screening campaign (Figure 1.). In addition, known 5-HT1AR ligands were redocked into this ensamble and obtained binding poses are in accordance with the modes described in literature.

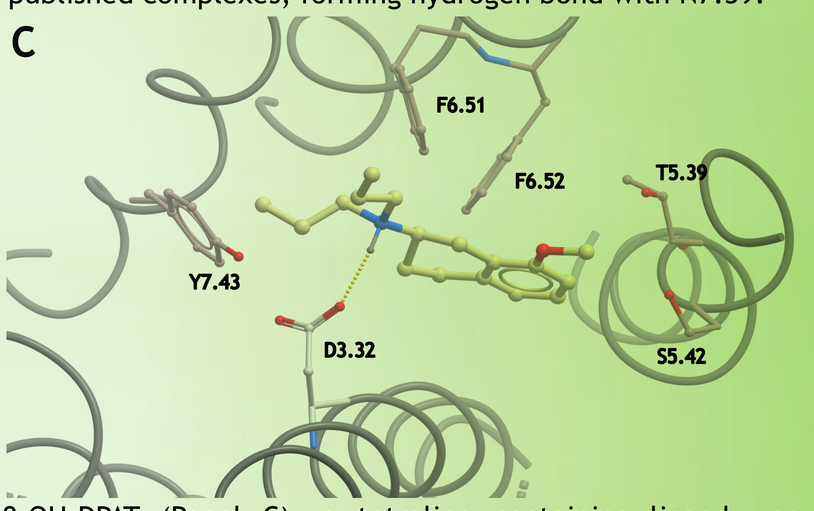
Binding mode of known ligands



Serotonin (Panel A), a representative of indole-like ligands was docked in accordance to the binding mode showed by Seeber [8] and interacted with TM3, TM5 and TM6. Strong, charge-assisted hydrogen bond is formed between NH_3^+ group and D3.32. OH group interacted with T5.39, however contacts with S5.42 were also detected. Indole moiety formed face-to-edge stacking with F6.52.



Buspirone (Panel B), a member of a vast group of arylpiperazines was docked in a similar way to the pose proposed by Bronowska [9] and Sylte [10]. Protonated nitrogen atom created a charge-assisted hydrogen bond with D3.32. Pyrymidine moiety was located between TM5 and TM6, but interactions with TM6 were weak. Azaspirone part was more directed towards TM7 than in already published complexes, forming hydrogen bond with N7.39.



8-OH-DPAT (Panel C), a tetraline containing ligand was docked in a pose similar rather to that proposed by Sylte [10] than the binding mode described by Seeber [8]. Tetraline moiety was perpendicular to the membrane surface and formed face-to-edge stacking interaction with the aromatic cluster from TM6. OH group interacted with TM5 (S5.42). Protonated nitrogen atom created charge-assisted hydrogen bond with D3.32, whereas n-propyl chains had contacts with Y7.43 and EL2.

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