New ligands identified for thrombin by virtual screening

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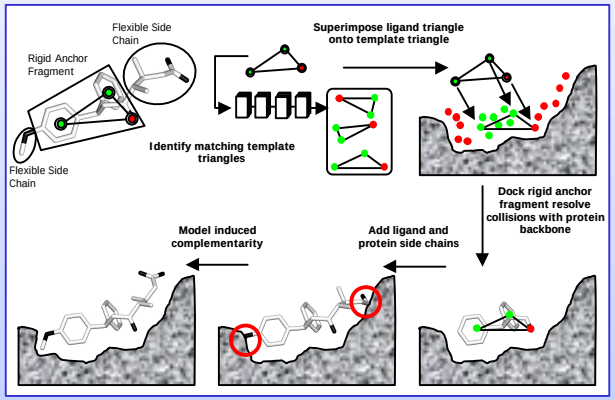
Screening for Ligands with Induced–fit Docking Efficiently

SLIDE is a computational tool developed in our laboratory[1] which can efficiently screen databases of hundreds of thousands of molecules identifying feasible ligand candidates for a target protein with known three dimensional structure.

The algorithm

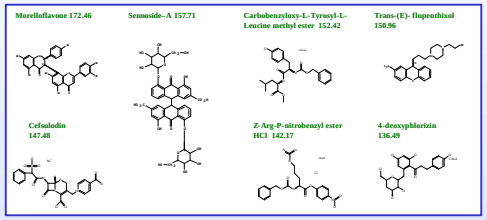


The Available Chemicals Database (214713 compounds) was screened to discover new ligands for thrombin*

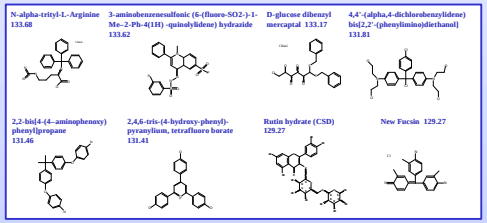
- **SLIDE returned 15474 molecules that fit into the active site of thrombin, with an average of two docked orientations /compound.**
- **Runtime: ~ 46 hr on a double–processor desktop workstation**
- **The top 3000 orientations were rescored with DrugScore[2], and ranked according to their consensus score, calculated as the normalized sum of their SLIDE and DrugScore score.**
- **15 compounds were chosen (7 based on score and 8 based on visual inspection of the top 300 dockings) for measuring their binding affinity by Isothermal Titration Calorimetry.**

***The screening experiment was performed in the laboratory of Dr. Elizabeth Getzoff and Dr. John Tainer at the Scripps Research Institute.**

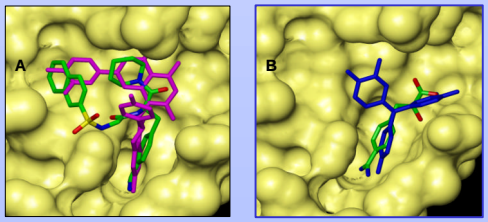
Compounds selected based on score



"Hand–picked" compounds

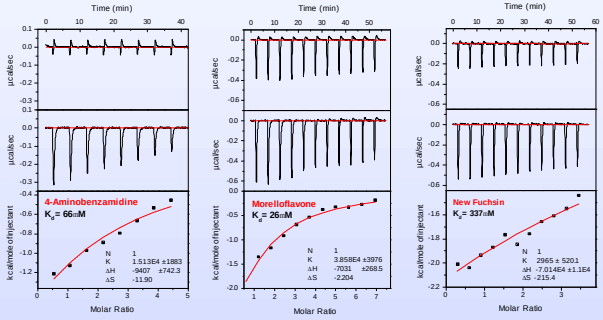


Of the 11 compounds that proved to be soluble, two showed micromolar binding affinity to human thrombin and are novel ligands for this protein: Morelloflavone and New Fuchsin.



Docked orientation of Morelloflavone (A) and New Fuchsin (B) in the binding site of thrombin. The molecules colored by atom types are known thrombin inhibitors from X–ray structures (PDB codes 1dwd [A] and 1aht[B], respectively).

ITC results for the positive control 4–Aminobenzamidine and the two new ligands, Morelloflavone and New Fuchsin.



The recorded heat changes upon successive injections of the ligand into the buffer (negative control) and the protein solution are shown on the two upper panels of each graph. The integrated heats plotted against the molar ratios are shown in the lowest panels. The red lines represent the least square fitting of the one–binding–site /protein model to the experimental data. The binding affinity is one of the parameters calculated from this fitting.

We achieved a hit rate of 18% when identifying new ligands for thrombin based on SLIDE virtual screening. This rate is comparable to the best results reported by other groups[3], and is 2000-fold more effective than *in vitro* high-throughput screening, which typically has a hit rate of ~0.01%. Furthermore, SLIDE explicitly predicts the binding mode between protein and ligand, which aids in optimizing the new ligands for higher affinity and protein selectivity.

References

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