

SynSPROUT – de novo ligand design with synthetic constraints



SynSPROUT addresses the problem of synthetic accessibility, that has historically plagued de novo ligand design, by using a set of user modifiable chemical reactions during the structure generation process within the constraints of the receptor site.

The de novo design process in SynSPROUT is a straightforward one. The user can choose to use the provided fragment databases and synthetic knowledge bases as the basis for the synthetic de novo design. Then the user is guided, via the graphical interface, through the process of setting up a receptor site, identifying interaction sites, docking start fragments and structure generation with synthetic considerations. SynSPROUT also provide the user with a suite of analysis tools for scoring, ranking, and viewing the results of the de novo design.

HIV-1 Case Study (1TCX):

In this example the HIV-1 triple mutant (V32I I47V V82I) protease complexed with SB203386 tripeptide analogue inhibitor (PDB code 1TCX) was chosen to verify SynSPROUT's capability to generate peptide-like inhibitors.

HIV-1 aspartic acid protease performs an essential cleavage reaction in the late life cycle of HIV virus. Thus blocking this action by tightly bound inhibitor prevents replication of the infectious virus. Therefore HIV-1 protease has emerged as one of the most studied enzymes for the development of anti-AIDS drugs, resulting in number of potent and selective inhibitors, most of whom are peptidic in nature.

Generation of amino acid library

First of all, a 3D amino acid library was generated from the 20 common amino-acid building blocks resulted in 305 low energy conformations. The corresponding peptide synthetic knowledge base, used for structure generation includes only the amide and disulphide formation rules.

Analysis of a 1TCX HIV-1 complex

HIV-1 is a small enzyme composed of two identical protein chains that embrace the active site. Figure 1 shows the active site with the interatomic distances of potential hydrogen bonds

between the inhibitor and the residues of the enzyme.

Accordingly, 5 heteroatoms of the inhibitor form hydrogen bonds

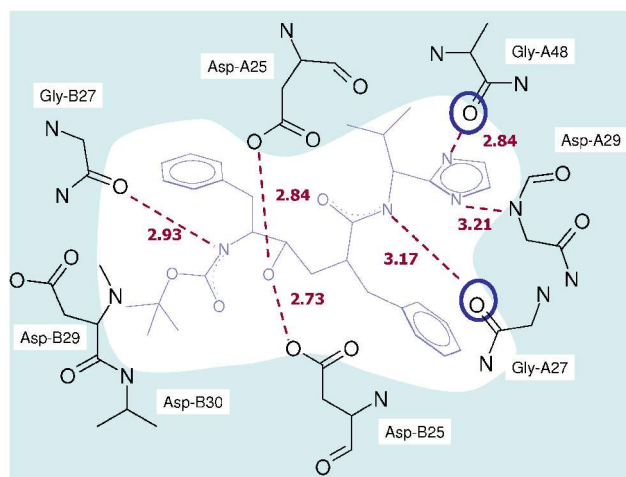


Figure 1: The binding pockets of HIV-1 protease with the bound SB203386 inhibitor, schematic representation of the 1TCX complex (dotted lines indicate potential hydrogen bonding interactions, while blue circles represent the only two donor sites which are indicated by HIPPO to be satisfied by the inhibitor)

that are essential for the tight binding for the enzyme. However, after generating 13 acceptor and 15 donor hydrogen sites for the enzyme in the HIPPO module, the automatic analysis of the enzyme-ligand interactions by HIPPO failed to identify four of these interactions and only two donor sites as hydrogen bonding sites satisfied by the inhibitor. These two donor sites are generated by the amide oxygens of the GLY-A27 and GLY-A48 residues.

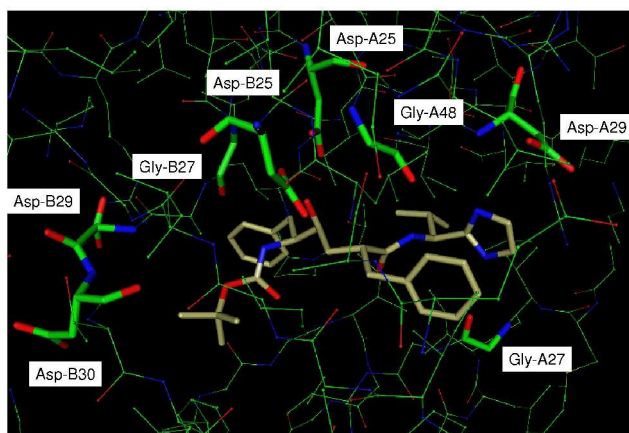


Figure 2: snapshot of the peptide analogue inhibitor with the most important residues

In the SynSPROUT run, 5 hydrogen target sites were chosen to be connected in the structure generation process. In addition to the two donor sites identified as satisfied (O GLY-A48 and O GLY-A27), three other sites are selected in order to explore alternative binding modes. These additional sites include one donor (OD2 ASP-A25) and two acceptor sites (N ASP-B29 and N ASP-B30) (see Figure 3).

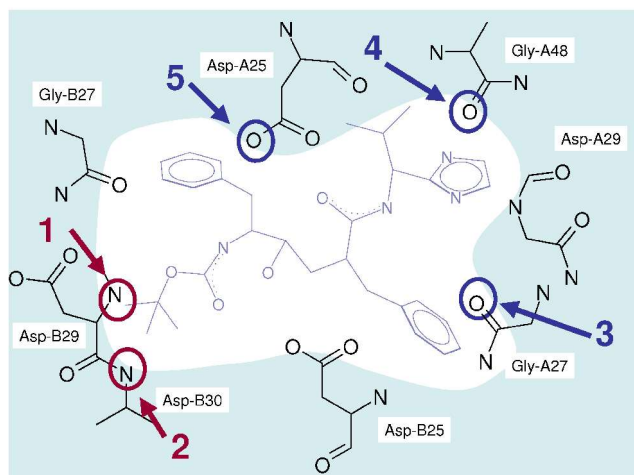


Figure 3: The key interaction region of HIV-1 with the target sites selected to be connected (red, and blue circles indicate the selected acceptor and donor target sites respectively).

ASP-B30) (see Figure 3).

Generation of peptide ligands.

In the initial step of the structure generation process, an attempt was made to dock each fragment from the amino acid library to the selected hydrogen sites. Due to the small distances between

target sites, N ASP-B29 acceptor site with N ASP-B30 and O GLY-A48 donor sites with O GLY-A27 are regarded as groups in the docking process.

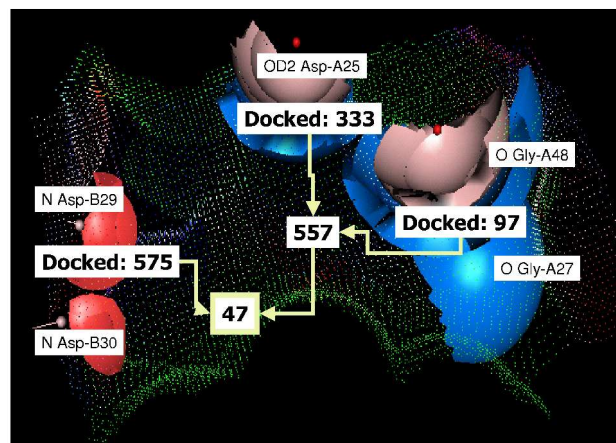


Figure 4: The connection of the selected target sites in ITCX (boxes and arrows indicate the steps of the connection).

The successfully docked amino acids making favourable interactions with the enzyme provide the starting point for the peptide ligand generation. The connection of the selected target

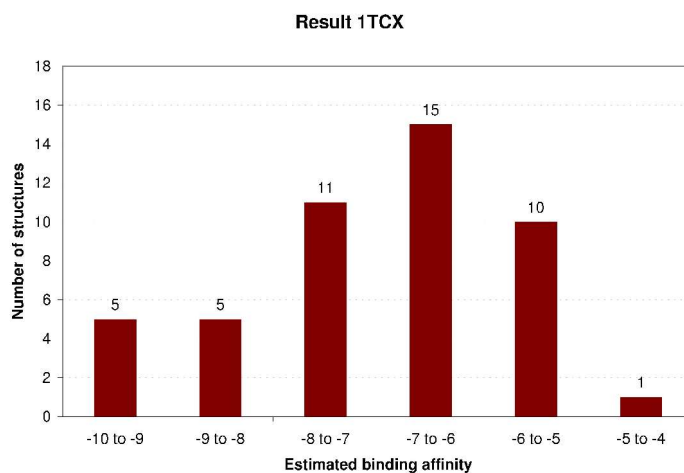


Figure 5: The distribution of the estimated binding affinity of the molecules generated for the ITCX complex.

sites took less than 1 hour elapsed time including the initial docking phase. The steps of the connection which resulted in 47 peptides satisfying all of the selected target sites are depicted in Figure 4.

The distribution of the estimated binding affinity (see Figure 5) reveals that the generated peptide structures have slightly worse

estimated binding energy than the original SB203386 tripeptide inhibitor. SynSPROUT estimates a score of -10.4 for the original inhibitor, while the best score for the generated ligands was -9.56 (see Figure 7).

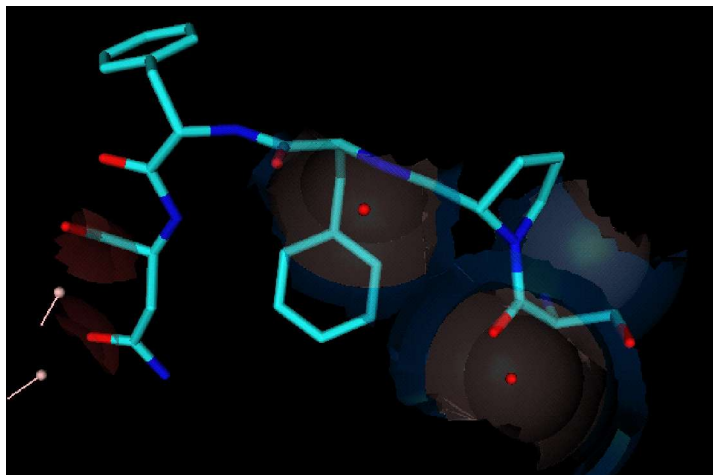


Figure 6: The best scored peptide ligand built by 4 amide formation reaction, SynSPROUT view.

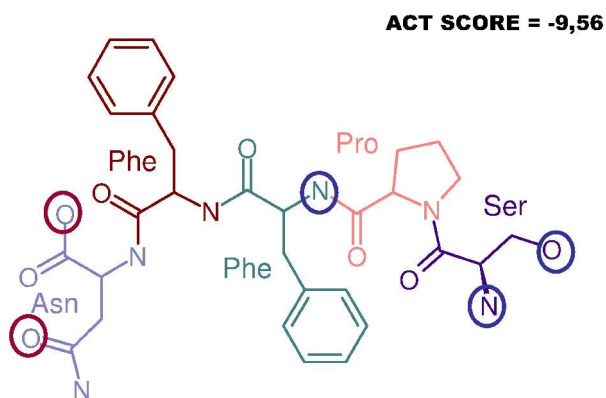


Figure 7: The best scored peptide ligand, 2D schematic, circles indicate ligand atoms docked to sites

Peptide structures generated for ITCX utilizing the amino acid library (cycles indicate ligand atoms docked to sites).

However, examination of the resulting structures shows that SynSPROUT proposed quite structurally diverse peptides (see Figure 8).

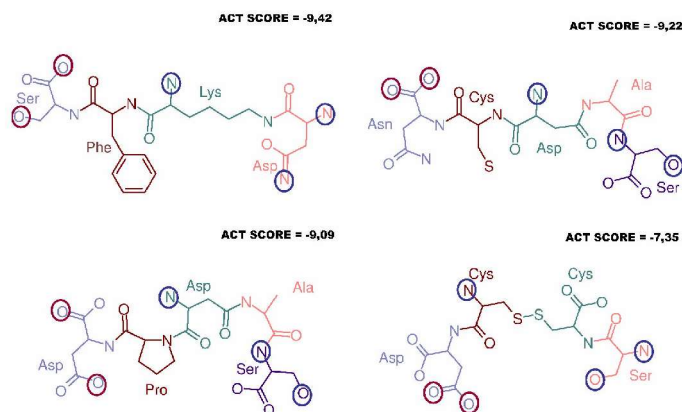


Figure 8: Peptide structures generated for ITCX utilizing the amino acid library

Additional Information

The above example illustrates SynSPROUT's ability to create peptidic ligands, however, SynSPROUT also contains a more general fragment library, extracted from the MDDR database of molecules. This library contains over 5000 template fragments and ~30 high yielding synthetic reactions and can be used to build more diverse ligands. In addition, SynSPROUT is able to work with user defined databases and user modified synthetic rules, and thus can be customized to work within the chemical space of interest.

For more information about SynSPROUT, or to request a free evaluation of the software please contact:

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