Summary

A critical point in modern drug design is the step from a pharmacophore hypothesis to a suitable lead structure. *In silico* docking methods[98] are used if the 3D structure of the receptor is available. In other cases one can resort to *de novo* design[11] or QSAR concepts.[85]

To support lead structure generation, a fragment based approach has been developed in the present work. It is based on a combination of *de novo* design, pharmacophore searching and docking.

A special advantage of the method developed here is that it can be applied to cases where x-ray structures of the receptor or of a complex of the receptor with a ligand are not available. The conformation of the ligand in the bound state can be obtained by transferred NOESY NMR, while the binding epitope can be elucidated by STD NMR. Based on these a procedure depending exclusively on ligand data of the can be developed.

Based on Bemis' and Murcko's publication[25] on the scaffolds of modern drugs, nine ring systems and five linkers have been selected as building blocks. In an *in silico* combinatorial process, controlled by a script in the SYBYL-programming-language SPL, these building blocks have been combined to form 225 ring-linker-ring systems.

On each ring system anchor atoms were defined for further linking. Since the primary aim has been the construction of ligands with suitable steric properties, all information on atom types was discarded.

To account for the high flexibility of the molecules, the conformational space for each was sampled by a systematic search along all torsion angles of each linker in steps of 10 degrees. The resulting 28.2 million rotamers were stored in a database. For all rotamers below an energy-cutoff of 1 kcal/mol, the minimum and maximum distance between pairs of anchor atoms was calculated and stored in a database to look-up potential leads. For these calculations, a program in the programming language C was developed. For an easy access to the database, appropriate tools in UNIX-shellscript and the SPL were developed.

To test the database-concept on a real problem, the interaction of human CD4 and HIV-GP120 was modeled. To obtain 3D-coordinates, methods of molecular modeling were applied to an x-ray-structure published by Kwong et al.[188] The binding epitope in question had been identified by Wülfken using STD-NMR[42] in 2001.[34]

Two hydrogens of GP120, one of the ε-CH_2-group of $Lysine_{429}$ and one of the δ-methyl-group of $Leucine_{125}$ were chosen as key functional groups for the GP120-CD4-interaction. Their distance was 11.5 Ångstrom. A database-search with this distance yielded 943 primary hits.

Using the DOCK program package[206] and a newly developed method named "constrained docking" 283 of these hits were rejected and an energy-ranking of the remaining 660 hits was obtained.

The five top scoring ligands were chemically functionalized *in silico*.

The ligands were modified at one of the two interaction-sites and were ranked with DOCK. The most favorable modifications at each site were combined in one molecule. As a proof of concept these molecules were docked "unconstrained" to CD4 and their binding energies were compared to the energies of the unmodified hits.

The addition of functional groups to the ligands improved the binding energies by up to 10 kcal/mol. The top scoring ligand (B2B5T2_L00K01) was composed of a naphthalene ring system which was linked with a two-bond-linker to an 1H-pyrrol which was modified by an ethylamino-group. The binding energy of this ligand was -18 kcal/mol.

This approach was successful in creating and modifying the scaffolds in a way that DOCK could place them in the pharmacophore-region with improved binding energies.

To verify these promising *in silico* results *in vitro* and to reach new nonpepdidic CD4-binding-ligands, some of the ligands proposed here will be synthesized and analyzed in the research group of Prof. Meyer in the near future.

The database that has been developed in this work is a new tool for *in silico* idea generation in lead structure design.