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On the use of LUDI to search the Fine Chemicals Directory for ligands of proteins of known three-dimensional structure

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SUMMARY

It is shown that the computer program LUDI can be used to search large databases of three-dimensional structures for putative ligands of proteins with known 3D structure. As an example, a subset of ≈30 000 small molecules (with less than 40 atoms and 0–2 rotatable bonds) from the Fine Chemicals Directory has been used in the search for possible novel ligands for four different proteins (trypsin, streptavidin, purine nucleoside phosphorylase and HIV protease). For trypsin and streptavidin, known ligands or substructures of known ligands are retrieved as top-scoring hits. In addition, a number of new interesting structures are found in all considered cases. Therefore, the method holds promise to retrieve automatically protein ligands from a 3D database if the 3D structure of the target protein is known.

INTRODUCTION

Structure-based design and discovery of protein ligands has recently emerged as a new powerful tool in medicinal chemistry [1–3]. Especially in the initial phase of a new drug design project, one is mostly interested in discovering novel lead structures. In view of the rapidly growing number of protein structures that are now available, methods that exploit the 3D structural information to search for novel ligands in 3D databases are becoming more and more important. One pioneering program belonging to this category is DOCK, developed by Kuntz and coworkers [4–8]. The basic idea of DOCK is to search a 3D database for possible ligands based on shape complementarity between protein and ligand. Recently, the ability to account for electronic complementarity was also incorporated by means of using a molecular mechanics force field [5,6]. A number of successful applications of DOCK have been reported [7,8]. Another program is CLIX [9], which uses the GRID force field [10,11] in the docking of putative ligands. The program was used to search for ligands of a mutant influenza-virus hemagglutinin.

We have recently reported on the development of a new computer program, LUDI, aiming at the de novo design of protein ligands [12–14]. This program positions molecular fragments or small molecules into protein binding sites in such a way that hydrogen bonds are formed with the protein and lipophilic groups are placed into hydrophobic pockets. The positioning of the ligands is guided by rules on favorable nonbonded contact patterns, derived from a statistical analysis of the Cambridge Structural Database [15–17]. In the present paper, we demonstrate that LUDI can also be used for searching large databases of more than 10 000 compounds for putative protein ligands.

In comparison with previous methods to search 3D databases for protein ligands, the present approach is believed to have a number of advantages. First, the docking of fragments in LUDI is based completely on geometric operations, without any use of force field calculations. This offers the possibility to search 3D databases very fast. Second, we recently described a novel scoring function that was fitted to experimentally determined binding constants of 45 protein–ligand complexes [18]. This scoring function takes into account both enthalpic and entropic contributions. Therefore, we expect a better prioritization of the hits.

The present paper outlines our effort to employ large databases for LUDI. The database employed is the Fine Chemicals Directory (FCD) [19]. All compounds collected in the FCD are commercially available. In the next section, we describe the technical details of the procedure to convert databases into a format suitable for LUDI. Then a number of applications are presented to illustrate the capabilities of this new approach.

METHODOLOGY

The computer program LUDI

The computer program LUDI has been described in detail previously [12–14]. The basic steps are:

(1) generation of the interaction sites;
(2) fit of molecules onto the interaction sites;
(3) evaluation of the resulting protein–ligand complex.

The first step is the generation of the interaction sites. These are positions in space suitable to form favorable interactions with the protein. The interaction sites are derived from rules that were described previously [13]. They are either points (describing lipophilic binding regions) or vectors (point pairs, used for polar binding regions).

The next step is the fit of the small molecules onto the interaction sites by rms superposition. This involves a superposition of atoms from the molecule onto the interaction sites. For technical reasons (mainly to keep the program fast) LUDI can at present fit molecules onto up to five interaction sites. We plan to remove this limitation in future versions of LUDI. We have developed a utility program, GENFRA, that analyzes the molecule and then selects automatically those atoms that can be employed in the fit. GENFRA first assigns atom types to every atom of the fragment. It uses the same atom types as described previously [13]. It then calculates a rough estimate of the accessible surface for every atom and removes those atoms from the list of potential centers for fitting which have accessibilities less than a threshold value (typically 1/3 of the complete surface of an isolated atom, assuming standard van der Waals radii). All lipophilic atoms directly bound to polar atoms are also removed from the list. The remaining atoms

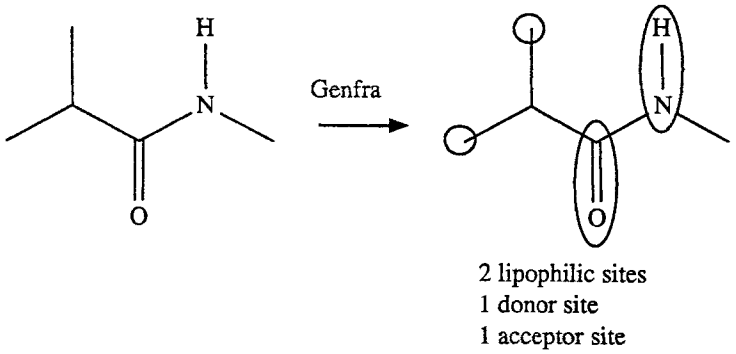

Fig. 1. Selection of fit sites.

are then used for the fit of small molecules. The procedure is exemplified in Fig. 1. A problem arises for large structures with a large number of possible fit centers. For example, 2,4-diaminopteridine contains six heteroatoms and could, at least in principle, form up to eight hydrogen bonds. Since LUDI can at present only handle up to five interaction sites, a selection has to be made. It should also be noted that not all polar atoms of a ligand are necessarily involved in direct interactions with the protein. Frequently, it is observed that the ligand is still partly solvent accessible, even when bound to the protein. Currently, we approach this problem by taking into account several different subsets of the atoms that are used. In order to speed up the 3D search, the number of permutations is limited to three at present. In certain cases, this may result in missing some of the larger fragments as potential hits. The present approach is also not without problems for very small fragments, such as acetamide (CH_3CONH_2). In the fitting process it is assumed that all polar groups are involved in direct interactions with the protein. However, one can easily imagine situations where this assumption is not strictly true, but nevertheless the fragments nicely complement structural features of the protein, as shown for example in Fig. 2. LUDI will miss this amide as an important hit. We are currently working on improvements both to LUDI and to GENFRA in order to cirumvent this problem.

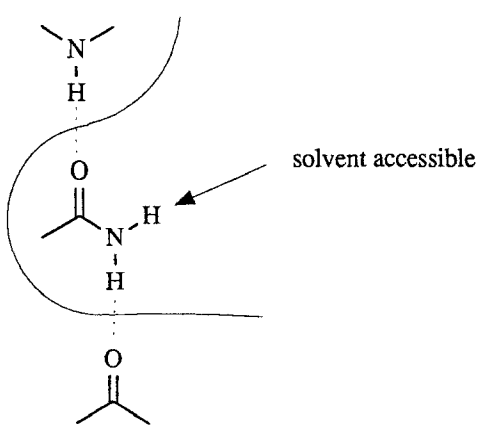

Fig. 2. Acetamide binding to receptor with one N-H group left accessible to the solvent.

The third step in the docking procedure performed by LUDI is the analysis of the resulting protein–ligand complex. An estimate of the free energy of binding is calculated using a simple empirical scoring function, which was described previously [18]. This scoring function was fitted to experimental binding constants of 45 protein–ligand complexes.

A problem arises from the choice of the protonation state of the ligand. As LUDI requires that the protons are specified, one has to define the protonation state of each ligand beforehand. In all the calculations reported in this paper, it was assumed that carboxylic acids, phosphates and sulfates are deprotonated and amines, amidinium and guanidinium groups are protonated.

In our search, we used one single 3D conformation per molecule, which was generated using the program CONCORD (version 2.9) [20]. There is a large number of structures in the FCD that contain several rotatable bonds and can therefore adopt several conformations. The use of one single conformer is a major limitation of the present approach and may result in missing some important hits. In order to circumvent this problem at least in part, we decided to restrict the current 3D search to the small molecules from the FCD, with less than 40 atoms and no more than two rotatable bonds, resulting in a final number of 30 000 structures used in the 3D search. The limit of two rotatable bonds was chosen in order not to exclude too many simple molecules with rotatable bonds, for example structures with ethyl substituents.

The docking procedure carried out by LUDI is based completely on geometric operations and interaction rules, with no force field calculations involved. A structure is considered as docked successfully into the protein binding site if the following criteria are met:

(1) There is no van der Waals overlap between the protein and the ligand. The allowed minimum contact distance between protein atoms and ligand atoms can be selected by the user (parameter VDWCUT).

(2) No electrostatic repulsion occurs between protein and ligand.

(3) A certain (user-defined) percentage of the ligand surface is buried after binding (parameter ICONMI).

(4) The actual score for the protein–ligand interaction exceeds a certain (user-defined) threshold value (parameter IMINSC).

(5) A certain (user-defined) percentage of the maximum possible score is obtained. The program calculates an estimate for the maximum possible score by assuming that the ligand is fully buried and all polar groups form ideal hydrogen bonds or ionic interactions with the protein.

(6) No unpaired (not involved in polar interactions with the protein) buried polar ligand atoms are found.

(7) No unpaired buried polar protein atoms are found that become buried upon ligand binding.

The set of input parameters that was used in the present calculation is given in Table 1. All ligands that fulfill the criteria listed above are scored using the scoring function described in Ref. 13 and are written to disk. After the completion of the 3D search, they are sorted according to their score.

In addition, it is now also possible to specify particular protein atoms that the ligand is required to form direct interactions with. This 'targeted mode' is useful if one wishes to exploit information from known ligands which are known to form certain essential interactions. The use of the 'targeted mode' can reduce the number of uninteresting hits obtained from a 3D search. It also speeds up the 3D search. In the present paper, the use of the targeted mode will be demonstrated with one example (HIV protease).

TABLE 1
INPUT FILE FOR THE LUDI RUN SEARCHING FOR TRYPSIN LIGANDS

Parameter	Value	Comment
TITLE		Search for ligands of trypsin
CUTOFF	4.0	Cutoff radius (Å)
XSITE	–2.190	Position of the center of search (x-coordinates)
YSITE	13.630	Position of the center of search (y-coordinates)
ZSITE	15.500	Position of the center of search (z-coordinates)
RMSMAX	0.70	Tolerated rms deviation of the fit
PRESEL	1.0	Preselection criterion
VDWCUT	3.0	Van der Waals overlap criterion
ESCUT	2.5	Electrostatic repulsion check criterion
ANGMAX	14	Not used (for link mode only)
IOUT	0	Minimum printout requested
IELEC	1	Electrostatic repulsion check is performed
IDENSL	35	Number of lipophilic interaction sites per protein atom
IDENSP	35	Number of polar interaction sites per protein atom
IFLAGV	1	No distinction between aliphatic and aromatic
ILINK	0	Run in standard mode
IANALG	0	Active-analog mode is not used
IBIFUR	1	Reject bifurcated hydrogen bonds
ICONMI	70	At least 70% of the ligand surface is buried
WLINK	1.0	Weighting factor
WLIPO	1.0	Weighting factor
WHBOND	1.0	Weighting factor
IRATMI	70	At least 70% of the possible maximum score is obtained
NFITS	9999	Maximum number of fit attempts per ligand
NHITS	900	Maximum number of hits
INEWSC	1	New scoring function is used [18]
IBURID	1	Check for buried unpaired polar atoms

RESULTS

In the following we report the results from 3D searches with LUDI for putative ligands of the proteins trypsin, streptavidin, purine nucleoside phosphorylase (PNP) and HIV protease. The top five are reported in all cases. In our test calculations, we have used a library consisting of 30 000 molecules with less than 40 atoms and 0–2 rotatable bonds from the FCD, as described above.

Trypsin

LUDI was applied to the search for ligands for the specificity pocket of the enzyme trypsin, crystallized as a complex with the inhibitor benzamidine [21]. The coordinates of the protein without inhibitor and water molecules were used as input (PDB entry 3PTB [22]). The hydrogen atoms of the amino acids were added using the program INSIGHT [23]. The amino acids aspartic acid, glutamic acid, lysine and arginine were assumed to be charged.

The calculation, using the large library, takes 118 min on a Silicon Graphics Indigo R4000 workstation and retrieves 153 compounds as potential ligands for trypsin. The five fragments with the highest score are shown in Fig. 3. LUDI calculates the highest score for *p*-methylbenzamidine. The second highest score is found for benzamidine, which binds trypsin with $k_i = 18$ μM [24]. *p*-Methylbenzamidine was indeed found experimentally to bind trypsin with a slightly higher binding

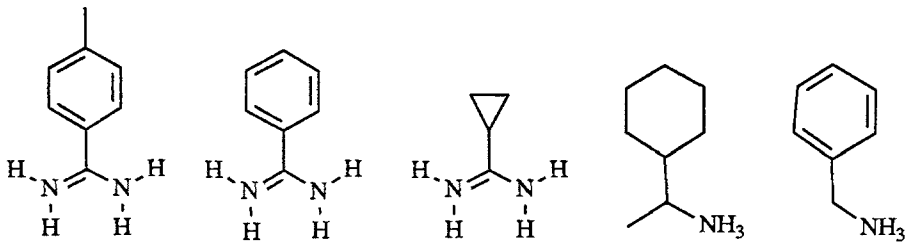

Fig. 3. Top five hits from a 3D search with LUDI in the Fine Chemicals Directory for putative ligands of trypsin.

affinity than benzamidine [25]. The predicted position of the benzamidine moiety is in very close proximity to the experimentally determined structure. The rms deviation of the fitted fragment from the benzamidine molecule found in the crystal structure (without hydrogen atoms) is 0.22 Å.

Streptavidin

As a second test case we have chosen the protein structure of streptavidin, complexed with biotin [26] (PDB entry 1STP [22]). The procedure was the same as for trypsin. The 3D search was restricted to the ureido binding site. The search took 160 min and retrieved 719 hits that match the required criteria. The five hits with the highest scores (all corresponding to log k_i(predicted) $< -5 \pm 1.4$) are shown in Fig. 4. The top scoring ligand is 4-methyl-2-imidazolidinone. This ligand was also found experimentally to bind streptavidin and avidin in the micromolar range [27]. The rms deviation of the fitted fragment 4-methyl-2-imidazolidinone from the corresponding moiety of biotin in the crystal structure is 0.80 Å. The ring is slightly shifted and tilted as compared to the X-ray structure. The rather large deviation is, at least partly, due to a very short hydrogen bond found in the crystal structure between the ureido oxygen and side-chain hydroxy group of Tyr^{43} ($R_{O\text{-}O}$ = 2.58 Å) which is shorter than the 'standard' hydrogen bond length of 2.9 Å, used by LUDI. The unsubstituted 2-imidazolidinone is also retrieved by LUDI. It is ranked as hit #9, still exhibiting a very high score (corresponding to log k_i(predicted) $< -5 \pm 1.4$), in agreement with available experimental data [36].

Purine nucleoside phosphorylase

A 3D search with LUDI was further applied to the protein purine nucleoside phosphorylase [28] (PDB entry 1ULB [22]). The search was restricted to the guanine binding site.

Interestingly, LUDI rejects guanine as ligand for PNP due to an unpaired buried amino group. A visual inspection of the X-ray structure of the PNP–guanine complex indeed reveals that the

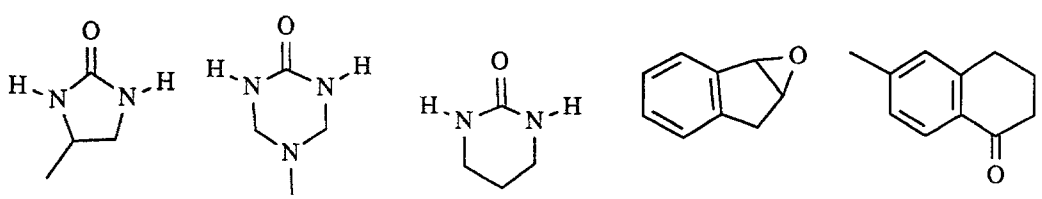

Fig. 4. Top five hits from a 3D search with LUDI in the Fine Chemicals Directory for putative ligands of streptavidin.

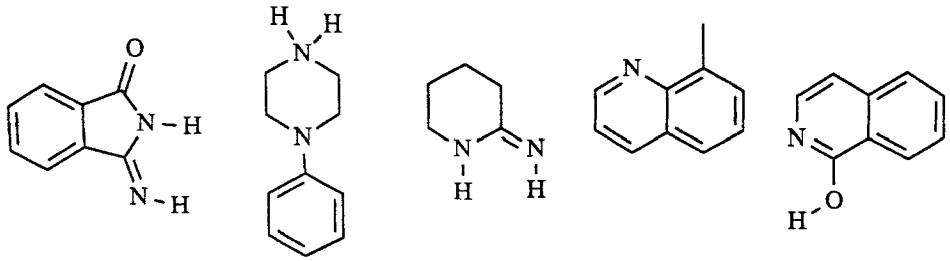

Fig. 5. Top five hits from a 3D search with LUDI in the Fine Chemicals Directory for putative ligands of purine nucleoside phosphorylase.

exocyclic NH_2 group forms one hydrogen bond with Glu^{201}, but the second proton is buried without forming a hydrogen bond with the protein. If the check for unpaired buried polar groups is turned off (IBURID = 0), then LUDI retrieves guanine as top scoring ligand for PNP. Guanine binds PNP in the low micromolar range [29].

Nevertheless, the 3D search in the FCD was carried out with the check for unpaired buried polar groups turned on (IBURID = 1), using exactly the same input parameters as in the previous examples. LUDI retrieves 187 ligands. The five hits with the highest score are shown in Fig. 5. The top scoring ligand is 3-iminoisoindolin-1-one; this is the only hit with a calculated score better than log $k_i = -5$. In the docked orientation, this molecule forms a hydrogen bonding pattern very similar to that of guanine.

HIV protease

As the final test case we have chosen HIV protease [30] (PDB entry 9HVP [22]). The procedure and the input parameters were the same as for the previous examples. The search was restricted to the catalytic site. In view of the rather open binding site of HIV protease, we decided to carry out two runs. The first run was performed using the same input parameters as for the previous examples. The second run was carried out in the targeted mode. In this run, the ligands were required to form hydrogen bonds with one of the catalytic aspartates and with the backbone nitrogen of Ile^{B50}. In most known HIV protease–inhibitor complexes the backbone nitrogens of Ile^{A50} and Ile^{B50} do not form direct hydrogen bonds with the inhibitor. However, the structures contain a conserved water molecule that forms two hydrogen bonds with the inhibitor and also hydrogen bonds to both Ile^{A50} and Ile^{B50}. Recently, the first HIV protease inhibitors have been disclosed that replace this water molecule and form direct hydrogen bonds with Ile^{A50} and Ile^{B50} [31]. The purpose of the second run was to exploit the possibility to search with LUDI for putative HIV protease inhibitors that form similar interactions.

The first run took 300 min and retrieved more than 500 hits from the FCD. The five top scoring hits (all with scores corresponding to log $k_i < -6 \pm 1.4$) are shown in Fig. 6. The top scoring hit, 1-amidino-2,5-dimethylpiperidine, is docked into the binding site in an orientation which allows the formation of an ionic interaction with the catalytic aspartates. None of the top scoring hits contains a hydroxy group interacting with the aspartates. Instead, LUDI very much prefers to position NH_2 groups in the vicinity of the catalytic aspartates. This is a result from the use of the scoring function described in Ref. 13, which scores an interaction with a positively charged N-H group and a negatively charged COO group higher than an O-H··OOC interaction.

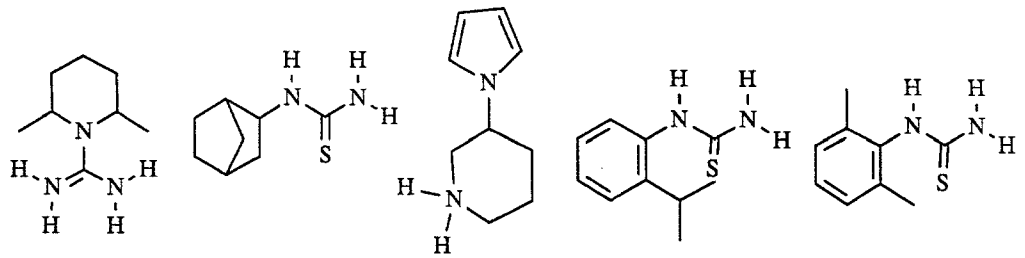

Fig. 6. Top five hits from a 3D search with LUDI in the Fine Chemicals Directory for putative ligands of HIV protease.

Indeed, potent inhibitors of aspartic proteases are known that interact with the catalytic aspartates via charged amino groups [32,33].

The second run, using the targeted mode, took 52 min and retrieved 21 candidate structures. The five top scoring hits from this run are depicted in Fig. 7. The structures are all docked in a very similar manner into the HIV protease binding site. The amino group should form a hydrogen bond with the catalytic aspartates and the acceptor group should hydrogen bond with Ile^{A50} and Ile^{B50}.

DISCUSSION AND CONCLUSIONS

The present paper describes recent progress in the development of the computer program LUDI which now makes it possible to search large fragment databases for protein ligands. The 3D search capabilities of LUDI are demonstrated for the proteins trypsin, streptavidin, PNP and HIV protease. The searched database was a subset of the Fine Chemicals Directory, consisting of 30 000 small rigid molecules. The 3D structures were generated with the program CONCORD [20]. The docked structures are scored using a novel scoring algorithm described previously [18].

For both trypsin and streptavidin the top scoring ligands that were retrieved in the 3D search have also been observed experimentally to bind the corresponding proteins in the low micromolar range. This lends some confidence to the results obtained from LUDI. In the case of PNP and HIV protease, the binding affinities of the top scoring ligands have, to our knowledge, not yet been determined.

A problem with all current 3D database search programs is that they can generate a potentially very large number of hits. One option is to review them all and to use chemical intuition to extract interesting candidate structures. This is a practicable approach if the number of hits is on the order of a few hundred. However, with the advent of de novo design programs and availability of very

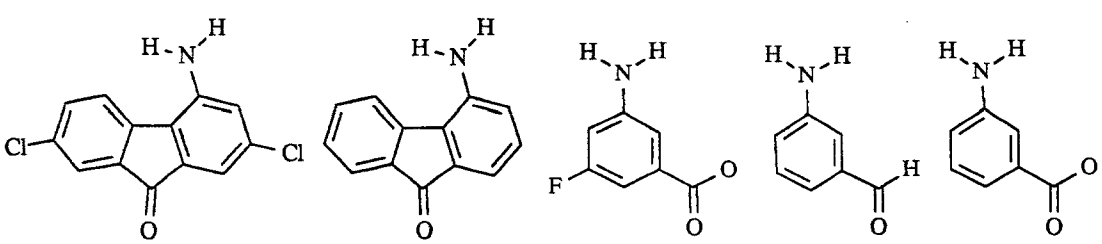

Fig. 7. Top five hits from a 3D search with LUDI in the Fine Chemicals Directory for putative ligands of HIV protease using the 'targeted mode'.

large databases, containing 10^6 or more structures, it is easily predictable that visual inspection alone becomes impracticable. Therefore, it is very important that useful candidate structures are found among the top scoring hits. It is very satisfying to note that in all four examples investigated in the present work interesting compounds are retrieved within the top five scoring hits.

In comparison with previous approaches [4–9], the 3D database search with LUDI has the following advantages. First, the present approach is very fast. In the examples, the search of a 30 000 compound database took between 60 and 300 min, corresponding to an average search time of 0.1–0.5 s per structure. It should be noted that LUDI considers a potentially very large number of different orientations per ligand in the docking process. For example, for the trypsin ligand benzamidine LUDI considers roughly 1 600 000 possible orientations in the docking process. However, only 83 orientations pass a rough geometrical check which is used to screen out potentially interesting orientations. Only those 83 orientations are then considered in more detail and an actual docking of the structure into the binding site is carried out. The vast majority of the 83 orientations is rejected due to overlap with the protein. The quite time-consuming scoring of the protein–ligand interaction (which takes about 0.1 s per ligand) is carried out only twice for benzamidine. Typically more than 90% of the structures in the database are rejected without any score being calculated. The second advantage of the present approach is the scoring function itself. This function was fitted to experimentally determined binding constants of 45 protein–ligand complexes [18]. It attempts to account for both enthalpic and entropic contributions to binding. It is believed that the scoring function is more accurate than previous approaches to the problem of the prioritization of the hits from a 3D search. In comparison with other approaches [4–9], one disadvantage of LUDI in searching large databases is its present limitation to small fragments with less than 50 atoms.

The applications reported in this paper also point to some limitations of the approach as currently implemented in LUDI. First, we note that the 3D search misses important structures that are contained in the FCD. For example, the FCD contains more amidines than found by LUDI, some of them known to be good ligands of trypsin. Second, there is the possibility that the program finds 'false positives' due to uncertainties in the scoring function. We also note that the number of hits strongly depends on the nature of the chosen problem. For very narrow binding sites a small number of high scoring hits is obtained, whereas for rather open binding sites, such as found in HIV protease, a much larger number of hits is obtained.

In summary, we have shown that the search of large 3D databases with LUDI is a very promising new approach to lead discovery.

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