Fluctuation Analysis and Accuracy of a Large-Scale *In Silico* Screen

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Abstract: Using a cascadic version of the stochastic tunneling method we perform an all-atom database screen over 186,000 flexible ligands of the NCI 3D database against the thymidine kinase receptor. By analyzing the errors in the binding energy we demonstrate how the cascadic technique is superior to conventional sequential docking techniques and how reliable results for the determination of the top-scoring ligands could be achieved. The substrate corresponding to the crystal structure used in the screen ranks in the upper 0.05% of the database, validating both docking methodology and the applicability of the scoring function to this substrate. Several high ranking ligands of the database display significant structural similarity with known substrates. A detailed analysis of the accuracy of the screening method is carried out, and its dependence on the flexibility of the ligand is quantified.

Key words: stochastic tunneling method; receptor ligand docking; global optimization; virtual database screening

Introduction

Virtual screening of a chemical database to targets of known three-dimensional structure is rapidly developing into a reliable method for finding new lead candidates in drug development.[1,2] Both better scoring functions[3] and novel docking strategies[4] contribute to this trend, although no completely satisfactory approach has been established yet.[5] This is not surprising, because the approximations that are needed to achieve reasonable screening rates impose significant restrictions on the virtual representation of the physical system.

Three mandatory ingredients of a reliable *in silico* screening approach, based on the direct approximation of the affinity in an all-atom force field, can be identified: (1) the docking algorithm has to find the global minimum of the potential (or free) energy surface within the given conformational space in an accurate and reproducible manner. (2) All relevant conformations of the receptor–ligand complex in nature must be accessible in the virtual representation of that system. Although ligand flexibility is now considered in many docking tools, the inclusion of receptor degrees of freedom is still in an experimental stage. (3) The scoring function, which approximates the free energy change from solvated isolated ligand and receptor to the complex must accurately approximate the experimental affinity.[5]

Present-day screening methods necessarily contain approximations for each of the above ingredients to permit the treatment of large ligand databases within acceptable time scales. In this investigation results for a cascadic docking technique, which reduces and permits to estimate fluctuations in the calculated binding energies in scoring large databases, are presented. With this technique a fixed receptor/flexible ligand screen using the stochastic tunneling technique[6,7] of the TK receptor against a database of nearly 200,000 ligands is performed, and the results are analyzed with respect to their binding energy fluctuations. It is found that 4 of 10 known substrates to the receptor score in the top 1% of the database, while the ligand corresponding to the receptor structure appears in the upper 0.05%. The analysis also reveals certain similarities between the known substrates and other high-scoring ligands.

This article is organized as follows: In the next section the docking method is described, followed by the scoring function and the ligand database. Then an optimization of the docking strategy is carried out to reduce the statistical fluctuations of the estimated affinities and the occurrence of false negatives in a database screen. The correlation function is employed to analyze the fluctuation characteristics of ligands in large-scale screening projects.

The ranking of 10 known substrates and the properties of some top scoring database ligands are discussed later.

Method

Docking Tool

The screens in this investigation were performed with *FlexScreen,* an all-atom docking approach[8,9] based on the stochastic tunneling method.[6] In this approach receptor and ligand are represented in atomistic detail, the global minimum of the energy surface is located using the stochastic tunneling method (STUN),[6] which allows the particle in the minimization process to "tunnel" through forbidden regions of the potential energy surface that is subject to a nonlinear energy transformation:

$$E_{\text{STUN}}(x) = \ln(x + \sqrt{x^2 + 1}). \tag{1}$$

Here, $x = \gamma(E - E_0)$. E_0 is the lowest minimum encountered by the dynamical process so far, and γ is a problem-dependent parameter that controls the steepness of the transformation. The form of the energy transformation was adapted to the docking problem[8,9]—the transformation in eq. (1) has no finite asymptote, and hence delivers a nonvanishing gradient even in regions of high energies.

Scoring Function

The following simple, first principle-based atomistic scoring function was employed:

$$S = \sum_{\text{Protein}} \sum_{\text{Ligand}} \left(\frac{R_{ij}}{r_{ij}^{12}} - \frac{A_{ij}}{r_{ij}^{6}} + \frac{q_i q_j}{r_{ij}} \right) + \sum_{\text{h-bonds}} \cos \Theta_{ij} \left(\frac{\tilde{R}_{ij}}{r_{ij}^{12}} - \frac{\tilde{A}_{ij}}{r_{ij}^{10}} \right), \tag{2}$$

which contains the empirical Pauli repulsion (first term), the Van de Waals attraction (second term), the electrostatic Coulomb potential (third term), and the angular-dependent hydrogen bond potential (term four and five). The Lennard–Jones parameters R_{ij} and A_{ij} were taken from OPLSAA,[10] the partial charges q_i were computed with InsightII and esff force field, and the hydrogen bond parameters $\tilde{R}_{ij}$, $\tilde{A}_{ij}$ were taken from AutoDock.[11]

The scoring function in eq. (2) is an approximation of the *in vacuo* binding energy of the ligand–receptor complex. Even in its simplest approximation the affinity of the ligand is the sum of this binding energy, the desolvation energy of the ligand and the desolvation energy of the receptor. The omission of the latter is appropriate for constricted receptor pockets in which all ligands with high binding energy displace essentially all water molecules. Because the focus of this study were the fluctuations of the binding energies rather than the affinities, desolvation energies of the ligands were neglected as well.
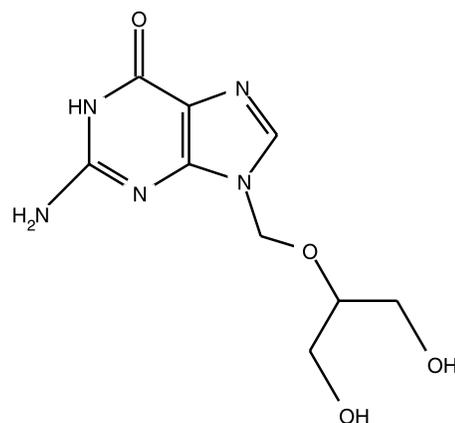
Figure 1. Chemical structure of ganciclovir (gcv), which consists of a rigid "body" (upper left) and a flexible "tentacle" (lower right).

Receptor Conformation

For this investigation the thymidine–kinase (TK) receptor in complex with its substrate ganciclovir (Fig. 1, pdb entry 1ki2[12]) was used as a model system. This system was used as a benchmark in several recent docking investigations[5,7,13] because crystal structures for 10 important substrates in complex with the receptor are available. Throughout this investigation the notation from ref. 5 is used. The protein was first protonated and partial charges were attached using InsightII and its *esff* forcefield. Crystal water molecules were removed, as was a sulfate–ion. The removal of the sulfate has been shown to influence the docking affinities of some substrates,[7] but its removal is nevertheless advisable in a large-scale database screen unless a proper treatment of solvation/desolvation effects is available and the flexibility of the position of the ion can be accounted for.

Ligand Database

The ligands were taken from the open part of the National Cancer Institute (NCI) database, *nciopen3D,*[14] which, in its latest version, contains 249,061 compounds and represents the largest freely available ligand database.[15] Not all compounds of nciopen3D are suited for *in silico* screening purposes: molecules with trivial file format problems and those in complex with exotic atoms (like rare earth metals), for which reliable force field parameters were unavailable, were removed. The remaining 239,887 compounds were processed with InsightII to attach the partial charges, again using the *esff* forcefield. This was successful for 238,739 compounds, 11,368 of which were not singly connected, i.e., composed of two or more molecules, and hence rejected, leaving 227,371 ligands suitable for screening. To introduce ligand flexibility, the molecules were investigated by an automated topological algorithm, which detects rotatable single bonds. These were enumerated as rotational degrees of freedom of the respective ligand. Exceptions were single bonds inside rings, bonds to trivial (single atom) end groups and conjugated bonds with delocalized π orbitals.

Figure 2 displays the distribution of the 227,371 ligands with respect to size and internal degrees of freedom (white area). For a
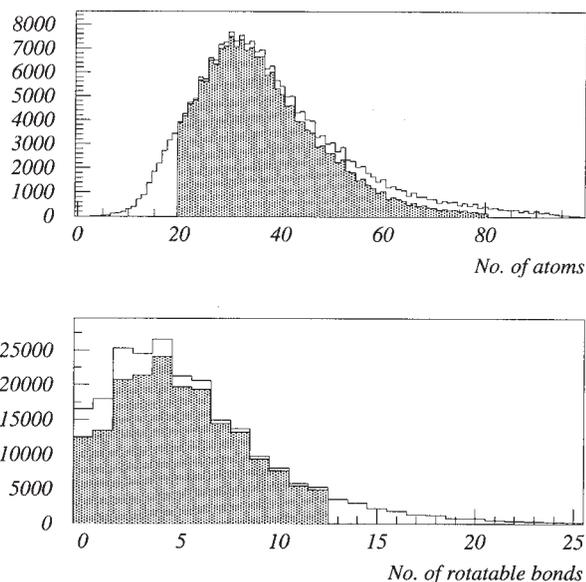
Figure 2. Distribution of compound size (upper panel) and number of internal degrees of freedom (lower panel) of the NCI (open) database. White area: 227,371 compounds that are singly connected and for which partial charges could be assigned. Gray area: 186,025 compounds used for the screen (see text for a description of their selection).

reasonable database screen, another filter was installed to select the admissible ligands: (1) the number of atoms N was restricted to $20 \leq N \leq 80$. Ligands with less than 20 atoms were to a large extent inorganic compounds that docked everywhere, that is, were too unspecific. Large molecules consisting of more than 80 atoms are unlikely to be useful as drugs, because their resorption rate is usually too low. (2) The number of rotational degrees of freedom was restricted to $R \leq 12$. It is obvious that a too flexible lead is losing its specificity, that is, its character as a key that fits into one specific lock. (3) Charged ligands were discarded. First, the inclusion of solvation effects would be required for a proper treatment of these ligands. Second, in many cases a residual charge turned out to be the result of InsightII's failure to assign consistent partial charges to the molecule. After this filter was applied, 186,025 ligands remained to be docked (gray area in Fig. 2).

Results

Reliability Analysis for a Single Ligand

To rank the ligands in a database, an estimate of their affinity must be found by global optimization of the scoring function. The accuracy of the screening procedure depends in large measure on the reliability of the docking algorithm. To illustrate the difficulties to determine the optimal value of the scoring function, a set of 300 independent docking simulations of 8×10^5 Monte Carlo steps each were carried out for gcv against its experimental receptor conformation. The global energy minimum could be estimated to

be close to -160 kJ/mol, the lowest energy found in any of the simulations, with an RMS deviation of 1.0 Å to the known crystal structure. Figure 3 displays the distribution of lowest energies found for the individual runs, which scatter over a wide range, indicating that a single scoring run is insufficient to reliably estimate the optimal score. There is, however, a good correlation between energy and the RMS deviation from the experimental conformation. The gray areas indicate conformations with less than 2.0 Å deviation from the X-ray structure. Using this threshold as a docking criterion, only 40% of the simulations led to a docked ligand, but even more of them failed to approach the optimal energy.

One obvious—and widely used—option to reduce the statistical noise is to repeat the scoring run several times, either with a corresponding increase in the computational cost, or, if feasible, with a reduced number of steps in each run. In this approach, the best balance between the number of runs and the length of the individual run must be found. It is difficult to calibrate the parameters on a single ligand, as docking performance depends significantly on the complexity of the conformational space. To illustrate the improvement obtained with this approach, eight docking runs of 10^5 steps were carried out in a batch, and the lowest binding energy obtained in any of these runs was selected. This simulation was repeated 300 times, and the resulting energy distribution (Fig. 3, center panel) was now clustered close to the global optimum and all conformations approached the experimental conformation.

To rationalize this result the docking rate was investigated as a function of simulation steps. When considering a single docking run (see Fig. 4), it can be observed that the STUN simulation
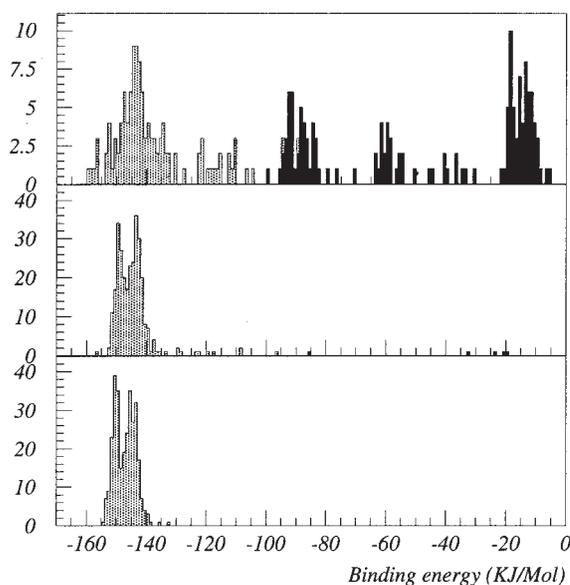
Figure 3. Histograms of the binding energy distributions for three strategies for docking gcv with the stochastic tunneling method. Upper panel: single runs with 8×10^5 MC steps each. Central panel: the best of eight runs of 10^5 MC steps. Lower panel: cascadic approach. Distribution of binding energies after 300 simulations. Gray area: successful docking. Black area: docking failures.
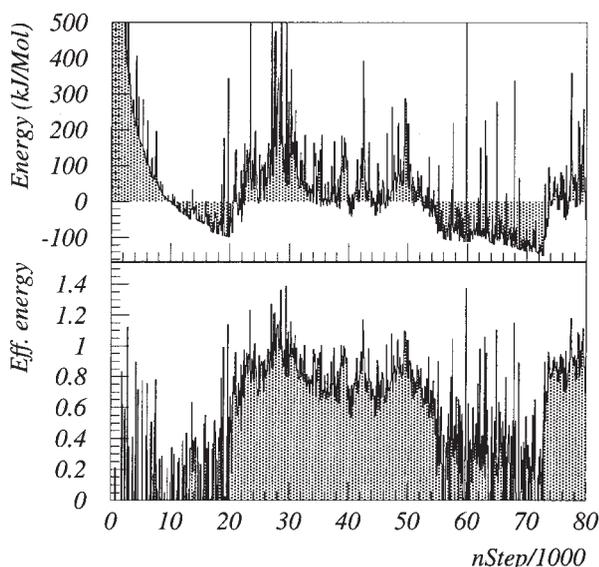
Figure 4. Energy (upper panel) and transformed energy (lower panel) for a representative docking simulation of gcv to its native receptor. Note that small fluctuations in the transformed energy, which serves as the target function of the dynamics process can lead to large changes in the original energy. The simulation alternates between local optimization and tunneling phases. The latter are essential to locate different metastable conformations.

continuously switches between local optimization and tunneling events. After each tunneling event the simulation explores a local minimum and then proceeds. In contrast to other methods, for example, simulated annealing,[16] the optimal configuration may be reached at any time during the simulation. It is therefore sensible to define the docking rate as the fraction of runs that had visited the optimal position at a given simulation step n. Figure 5 shows the docking rate in relation to the number of MC steps. There is an
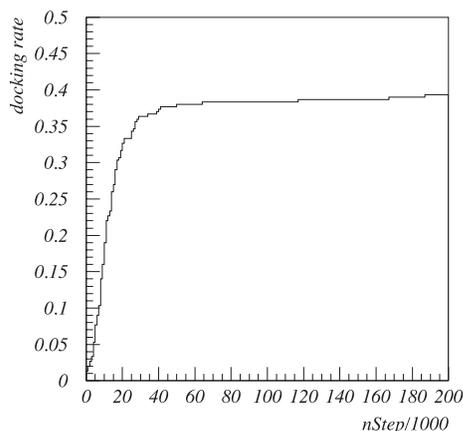
Figure 5. Docking rate for gcv as a function of the number of steps in the simulations. Shown is the fraction of runs (out of 300) that had visited the experimental conformation to within 2 Å rms deviation within the specified number of steps.

Table 1. Statistical Properties of the Distribution of Binding Energies (in kJ/mol) for the Different Docking Strategies as Shown in Figure 3.

Docking strategy	Failure (%)	Mean	RMS
$1 \times 8 \cdot 10^5$ steps	61.0	−88.4	52.5
$1 \times 8 \cdot 10^5$ steps	1.7	−142.9	15.6
Cascadic	0.0	−147.1	3.5

initial steep increase in the docking rate, but the curve flattens after approximately 50,000 steps, implying that some runs had visited optimal or near optimal conformations quite early in the simulation, but 60% of them never came close to the docked conformation.

This observation motivates a cascadic docking approach: the total number of simulation steps is divided into several partitions of similar computational effort. The first partition is spent by performing a large number of short runs on the ligand. Then a small fraction of well scoring runs from this set is selected for the second partition, where they are extended with a larger number of steps. This process is iterated, with decreasing number of conformations, but increasing number of steps. As a result, the most promising simulations at the end of each partition are allotted the largest computational effort, while unsuccessful simulations are terminated early.

To investigate this strategy, three partitions of 500,000, 150,000, and 150,000 steps, with 100, 5, and 2 simulations were allotted, respectively. Using this cascade, again 8×10^5 simulation steps were invested for each of the 300 ligands, but with this approach the success rate increased to 100% and the scattering of the final affinity was further reduced (see bottom panel of Fig. 3). Table 1 summarizes the statistical properties of the final binding energies. It is this reduction of the affinity fluctuations that is of particular importance when it comes to avoid false negatives in a database screen.

Database Screen and Fluctuation Analysis

If a large database has to be screened, a detailed investigation of energy fluctuations of individual ligands, as described in the previous section, is no longer feasible, because it would require a repeated docking of the entire database, or at least of a substantial subset. The cascadic approach introduced above permits an inherent assessment of the quality and reliability of the screen without additional cost by using energies of the final configurations of the last set of runs in the cascade to estimate the variance of the energy distribution.

In this database screen, the 186,025 ligands of the filtered database (see before) were screened with the cascadic technique as described earlier. The evaluation was confined to ligands that had a negative binding energy and either (a) reached an optimal position with a center of mass coordinate within 0.5 nm radius of the center-of-mass coordinate of the docked gcv substrate or (b) if the center-of-mass coordinate was outside this sphere, but at least 20 atoms were within. About 25.2% of the database molecules satis-
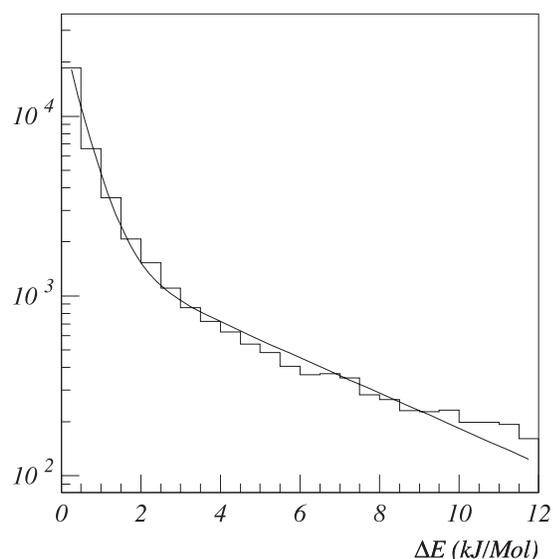
Figure 6. Distribution of the energy difference of the terminal energies in the cascadic approach on a logarithmic scale. The solid line shows the fit according to eq. (3). Two different approximately exponential regions appear for $\Delta E < 2$ ($\lambda^{-1} \approx 0.47$) and $\Delta E > 2$ ($\lambda^{-1} \approx 4.4$).

fied either (a) or (b), most others were simply too large or too inflexible to fit the cavity.

Figure 6 shows the distribution of energy differences ΔE of the two final runs in the above screening procedure. Its median ($\Delta E_m = 0.72$ kJ/mol) could serve as an estimate for the ligand's average energy uncertainty of the screening procedure. Alternatively, ΔE could be interpreted as a parameter of the energy–energy correlation function $F(\Delta E)$. In a fluctuation analysis, it was fitted as a sum of two negative exponentials, i.e.,

$$F(\Delta E) = w_1\lambda_1 e^{-\lambda_1\Delta E} + w_2\lambda_2 e^{-\lambda_2\Delta E}, \qquad (3)$$

yielding the inverse decay constants $\lambda_1^{-1} = 0.47$ kJ/mol, $\lambda_2^{-1} = 4.42$ kJ/mol, and weight factors $w_1/w_2 = 1.70$, with errors on the 5% level. The fit suggests that the energy fluctuations were composed of two classes of ligands: while some ligands docked with high reliability in the 0.5 kJ/mol range, others displayed significantly larger fluctuations of the order of 5 kJ/mol. For further analysis, the ligands were categorized according to their fluctuations: ligands with small fluctuations (SF-ligands) were those in the range of thermal fluctuations, ($\Delta E < 2.5$ kJ/mol $\approx k_B T$, 73% of the docked population), moderate fluctuations (MF ligands, 2.5 kJ/mol $\leq \Delta E < 10$ kJ/mol, 16%) or high fluctuations (HF ligands, $\Delta E \geq 10$ kJ/mol, 11%).

In Figure 7, the principal statistical properties of these families of ligands are summarized. A first striking feature is the fact that practically no ligands with more than 40 atoms have docked (upper left). The reason is that the TK receptor forms an almost entirely enclosed pocket. Ligands larger than a certain size were unable to fit the cavity (the substrate's sizes ranged from 25 to 37 atoms). A small systematic shift towards larger size is visible when compar-

ing SF and MF ligands with HF ligands. This trend was much more pronounced when the internal rotational degrees of freedom were considered (upper right). The family of SF ligands was dominated by ligands with few degrees of freedom. This is understandable, because an increasing number of degrees of freedom correlates with the complexity of the conformational space, making it more difficult for the optimizer to locate the global energy minimum within a given number of simulation steps. The energy distribution (lower left) indicates that ligands with high affinities were dominated by MF and HF ligands, i.e., those with less reliable docking results, which is explained with the correlation between the number of internal degrees of freedom and the binding energy (lower right): ligands with more rotational bonds reached lower energies than those with less degrees of freedom. A binding energy of lower than -200 kJ/mol was reached only by compounds with seven to nine rotational bonds (with high content of MF and HF ligands), whereas rigid ligands remained above -140 kJ/mol, and ligands with less than six rotational bonds remained above -170 kJ/mol.

A quantitative analysis of the energy fluctuations and their dependence on internal degrees of freedom is gained with a separate analysis of ligand families selected according to their degree of flexibility. In Table 2, the median errors, the estimated inverse
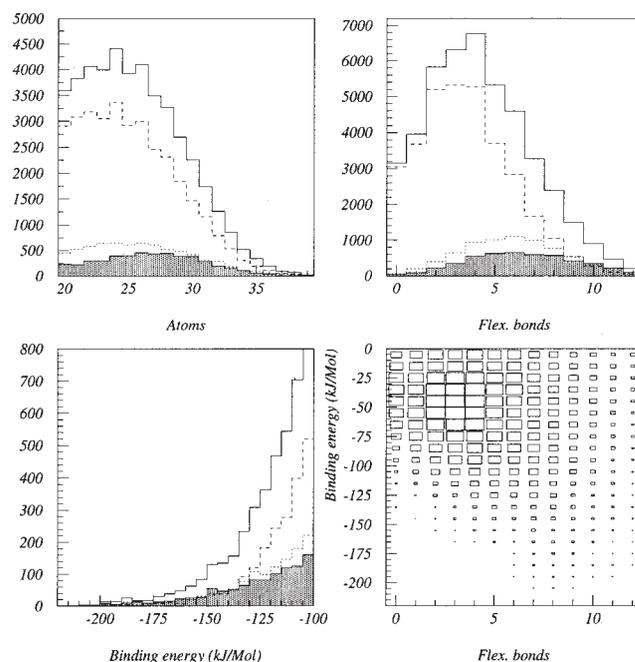
Figure 7. Histograms of the statistical properties of the docked ligands, the horizontal axis indicate the number of atoms of docked ligands (upper left), the number of internal degrees of freedom (upper right), the distribution of binding energies (lower left), and the an area plot correlating the binding energy with the number of internal degrees of freedom (lower right). In the 1D plots the solid line indicates the total number of ligands, while dashed, dotted lines, and gray areas indicate small-fluctuation (SF) moderate-fluctuation (MF) and high-fluctuation (HF) ligands, respectively. In the box plot, the area of the rectangle is proportional to the number of ligands in the corresponding bin.

Table 2. Median Energy Difference (kJ/mol, Exact), Inverse Decay Parameters λ_i^{-1} (kJ/mol) and Weight Factors w_1/w_2 (Errors: 5%) for a Fit of eq. (3) to the Correlation Functions of the Final Energies in the Cascadic Approach.

Flex. bonds	0–12	0–2	3–5	6–8	9–12	9–12(a)
Median	0.72	0.25	0.71	2.13	5.46	1.80
λ_1^{-1}	0.47	0.32	0.60	1.1	4.4	0.88
λ_2^{-1}	4.42	3.6	4.3	5.9	n.a.	7.3
w_1/w_2	1.7	7.9	2.4	0.9	n.a.	0.9

In the domain of 9–12 flexible bonds a single exponential fit was performed.
[a]Doubled number of steps.

decay parameters λ_i^{-1} and weight factors w_1/w_2 for different classes of ligands, grouped by the number of internal degrees of freedom, are shown. As is clearly visible, the uncertainty in affinity is increasing with the dimension of the conformational space. Although the median energy difference gives an overall estimate of the variance in the different families, the inverse decay parameters and their weights provide an individual "fingerprint" of the fluctuation characteristics of the screen. This analysis confirms that the majority of ligands with 0–5 flexible bonds fell into the category of reliably docking compounds ($\lambda^{-1} \approx 0.3$–0.6 kJ/mol, $w_1/w_2 > 1$).

The subset of compounds with 9–12 flexible bonds could be fitted with a single exponential function, yielding $\lambda^{-1} = 4.4$ kJ/mol. This indicates that the fast decaying component had actually vanished, leaving compounds with large fluctuation characteristics. At this stage of a screening project one may decide that the fluctuations of this particular class of ligands were intolerable, because they contributed unproportionally to the uncertainty of the overall screen. These ligands were then screened once again, with twice the number of simulation steps. The last column of Table 2 contains the results. The accuracy had now reached the accuracy level of the subset with 6–8 flexible bonds.

Analysis of the Top Scoring Ligands and Known Substrates

The top scoring compounds were rather simple, linear molecules with high flexibility (seven to nine rotatable bonds) and highly polarized end groups. The best among them reached a binding energy of −217 kJ/mol. These ligands are not likely to be of interest in pharmaceutical applications. Because of their high flexibility they can be accommodated in virtually any receptor pocket, thus lacking the specificity and structure to mediate biological function. Such a lead usually consists of one rigid part, which forms the "key," and perhaps some polarized and flexible extensions, which, like tentacles, help to catch hold on the receptor's side chains (Figs. 1 and 8).

As an attempt to satisfy these requirements, the database was filtered once more to select ligands which contained at least one ring. Despite of the fact that this subset formed the major part of the database (175,623 compounds or 94.4% of the total), the best ligand reached a binding energy of only −173 kJ/mol, and was ranked on position 99 of the original screen, indicating that it was strongly biased towards chain-like, flexible, ligands.

To analyze the ranking of the 10 TK substrates, each ligand was docked 64 times, their mean affinities and rms-deviations were used to determine the range of ranks within both the filtered and unfiltered database (Table 3). The resulting scores are reminiscent of earlier screening studies with smaller databases.[5] A few of the substrates were ranked high up within the upper 1% of compounds (gcv, acv, dhbt, hpt), while others still docked in the upper 20% of the database (ahiu, dt, idu, pcv), but would not be identified as potential lead candidates using this virtual database screen. Others (hmtt, mct) never docked at all. If only compounds with rings were accounted for, the rank of the well scoring substrates was significantly improved, because many fortuitous ligands with high affinity were removed.

Several of the top scoring ligands (with ring) displayed similarities to some of the known substrates. One example is shown in Figure 8, compared to substrate hpt. The last row in Table 3 contains the docking results of this compound, with a high rank and very low fluctuations. This result could be seen as an indication for the accuracy and high specificity of the cascadic docking approach based on the stochastic tunneling technique. One has to consider, however, that the fixed receptor conformation has generated a bias of the screen towards the natural substrate. As has been argued elsewhere, the restrictions imposed by receptor rigidity[7] and shortcomings of the scoring function[5] are among the main obstacles against an unbiased ranking of a diversity of ligands.
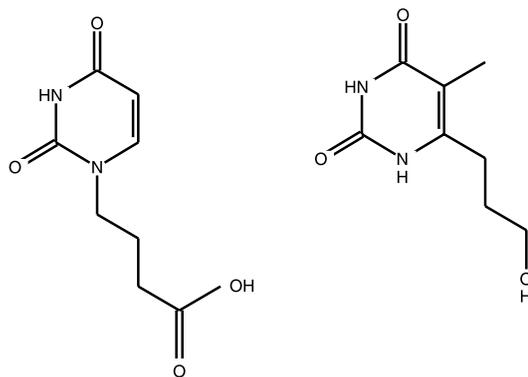
Figure 8. One of the top ranking database compounds (left) and the substrate hpt (right).

Table 3. Ranks of the 10-tk Substrates and One of the Top Scoring Leads When Screened Against the Unfiltered and Filtered Database (186,025 and 175,623 Compounds, Respectively).

Substrate	Rank (total)			Rank (rings)			Energy and RMS (kJ/mol)		N_a	N_f
	Mean	Min	Max	Mean	Min	Max				
gcv	421	352	522	93	69	131	−147.1	3.5	31	7
acv	463	354	586	113	68	156	−145.6	4.6	27	5
dhbt	990	846	1175	339	271	424	−130.3	3.0	29	7
hpt	1389	1211	1613	553	440	684	−123.8	3.0	25	5
pcv	10433	5543	17448	7330	3465	13188	−78.2	16.3	33	7
dt	21206	20305	22157	16445	15683	17298	−54.6	1.8	31	5
idu	24846	20152	20619	19653	15549	23858	−47.6	9.1	28	4
ahiu	33559	31101	36040	27291	25176	29426	−31.4	4.4	31	4
hmtt		nd							37	6
mct		nd							33	7
Fig. 8	166	157	175	14	13	15	−164.5	1.0	24	5

Each ligand was docked 64 times, the rms deviation served as an estimate for the range of ranks. N_a/N_f designate the number of atoms and ligand degrees of freedom respectively (nd indicates nondocking ligands).

Characteristic is the fact that gcv was among the best scoring ligands: as described earlier, the receptor conformation (pdb: 1ki2) was experimentally determined in complex with gcv, and is therefore optimized for this particular substrate. The bias in the receptor conformation is large enough to prevent a rather bulky substrate like hmtt from fitting into the pocket at all. On the other hand, the high score for gcv suggests that the scoring function Equation (2) was of reasonable accuracy. The analysis of the energy fluctuations indicates that the low scores of other substrates were not caused by inaccuracy of the optimizer.

Summary

In this investigation a full database screen over 186,025 ligands of the NCI (open) database using the stochastic tunneling method with a cascadic docking approach, which proved more reliable than competing sequential methods, was carried out. The cascadic docking approach optimizes the use of the available computational resources by concentrating on the best partial simulation in each run and permits a straightforward estimate of the variance of the binding energy of an individual simulation. It was demonstrated how the affinity fluctuations were reduced, and hence, the number of false negatives in docking simulations.

The analysis of correlation functions revealed a systematic dependence of the docking accuracy on the number of flexible bonds of the compounds. Based on these results, compounds with more than eight internal degrees of freedom were docked with additional computational effort to provide a reasonable quality of the database screen. It is desirable to identify critical families of ligands in an earlier stage of the project, i.e., in advance of a screen of the entire database. It therefore appears advisable to adopt the following strategy for a large-scale database screen (Fig. 9): first a representative subset of the database is screened and the fluctuation pattern is analyzed. Based on these preliminary results, an upper limit for the tolerable error in affinity is determined and
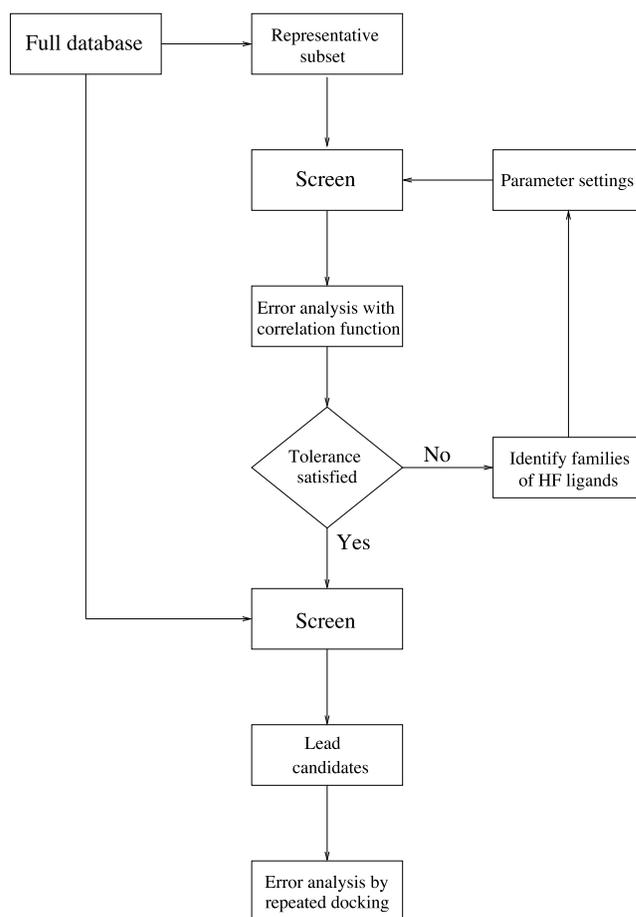
Figure 9. Flow chart for a quality control in large scale docking projects: the error analysis is carried out on two levels: (1) on the bulk statistical level, using correlation analysis to reduce the fluctuations of the background. (2) On the level of individual lead candidates, to estimate the accuracy of their rank against the background.

families of ligands are identified, which systematically exceed this limit. These are then rescreened, with a modified set of simulation parameters or simply with increased number of steps, until the required accuracy has been achieved. Next, the entire database is screened using the respective sets of parameters. Finally, the most promising leads among the top scoring ligands are selected. Due to fluctuations of their binding energies their ranking is uncertain. For an error analysis, these selected leads are screened repeatedly and the width of their affinity distributions are employed to bracket the range of ranks.

The investigation of the docking results was performed using both the entire database and a subset, where only molecules with rings were taken into account. Some of these top scoring compounds displayed a surprising degree of similarity to some of the 10 known substrates of the receptor. The substrate, corresponding to the protein conformation used in the screen ranked at position 93 in the upper 0.05% of the database, validating both the docking method and the scoring function used in this investigation. Several other known substrates also docked in the upper 1% of the database, while others failed to dock.

Based on the results of earlier investigations into the same receptor it was argued that the failure of some substrates stems from uncertainties in the scoring function and the absence of receptor flexibility. A characteristic signature is the fact that the substrate gcv, to which the receptor conformation was optimized, scored best among the substrates.

These results point to both strength and weaknesses of the all-atom docking approach using scoring functions based on physical interactions: the docking algorithm investigated in this study permits a reliable screening of databases with significant chemical variety with presently available computational resources. The top-scoring ligands bear significant resemblance to known substrates, but the screen proved highly selective for only four of the known substrates. Remaining shortcomings are the accuracy of the available scoring functions and the restrictions on the conformational space imposed by a rigid receptor geometry. Efficient docking methods, such as the cascadic stochastic tunneling method investigated here, will permit the relaxation of theses requirements and the systematic improvement of the scoring functions in the foreseeable future.

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