High-throughput docking for lead generation

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Recent improvements in flexible docking technology may lead to a bigger role for computational methods in lead discovery. Although fast and accurate computational prediction of binding affinities for an arbitrary molecule is still beyond the limits of current methods, the docking and screening procedures can select small sets of likely lead candidates from large libraries of either commercially or synthetically available compounds.

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Current Opinion in Chemical Biology 2001, 5:375–382

1367-5931/01/$ – see front matter

Abbreviations

ACD	Available Chemicals Directory
ER	estrogen receptor
HTS	high-throughput screening
ICM	Internal Coordinate Mechanics
MM	Mining Minima
PMF	Potential of Mean Force
QSAR	quantitative structure/activity relationship
RMSD	root-mean-square deviation
SEED	Solvation Energy for Exhaustive Docking
TK	thymidine kinase
VLS	virtual ligand screening

Introduction

Virtual ligand screening (VLS) based on high-throughput flexible docking is an emerging technology for rational lead discovery based on receptor structure [1,2]. Rapid accumulation of high-resolution three-dimensional structures, further accelerated by the structural proteomics initiative [3,4] and the improvements of docking and scoring technology, are making VLS an attractive alternative to the traditional methods of lead discovery. VLS can sample a virtually infinite chemical diversity of drug-like molecules without synthesizing and experimentally testing every screened molecule. Typically, a corporate high-throughput screening (HTS)-ready compound library ranges from 200,000 to 1,000,000 molecules. Even with corporate libraries as large as these, however, the experimental HTS often does not result in viable leads (Martin Rosenberg, personal communication). The high cost of such massive experimental testing and its technical complexity are further motivation for the theoretical alternative. Finally, the virtual experiment, as opposed to a high-throughput assay, can be easily designed to select for a particular binding site or receptor specificity. A flow chart of the flexible docking and VLS procedure is given in Figure 1.

Docking and screening methods have a long history that is described elsewhere (for a review, see [5,6]). We have not tried to cover docking and binding energy-prediction methods that are too computationally expensive for high-throughput applications and take more than 3–5 minutes per ligand per processor. Here we will review the most recent advances in the area of high-throughput flexible docking and computer screening, as well as applications of these techniques to lead discovery.

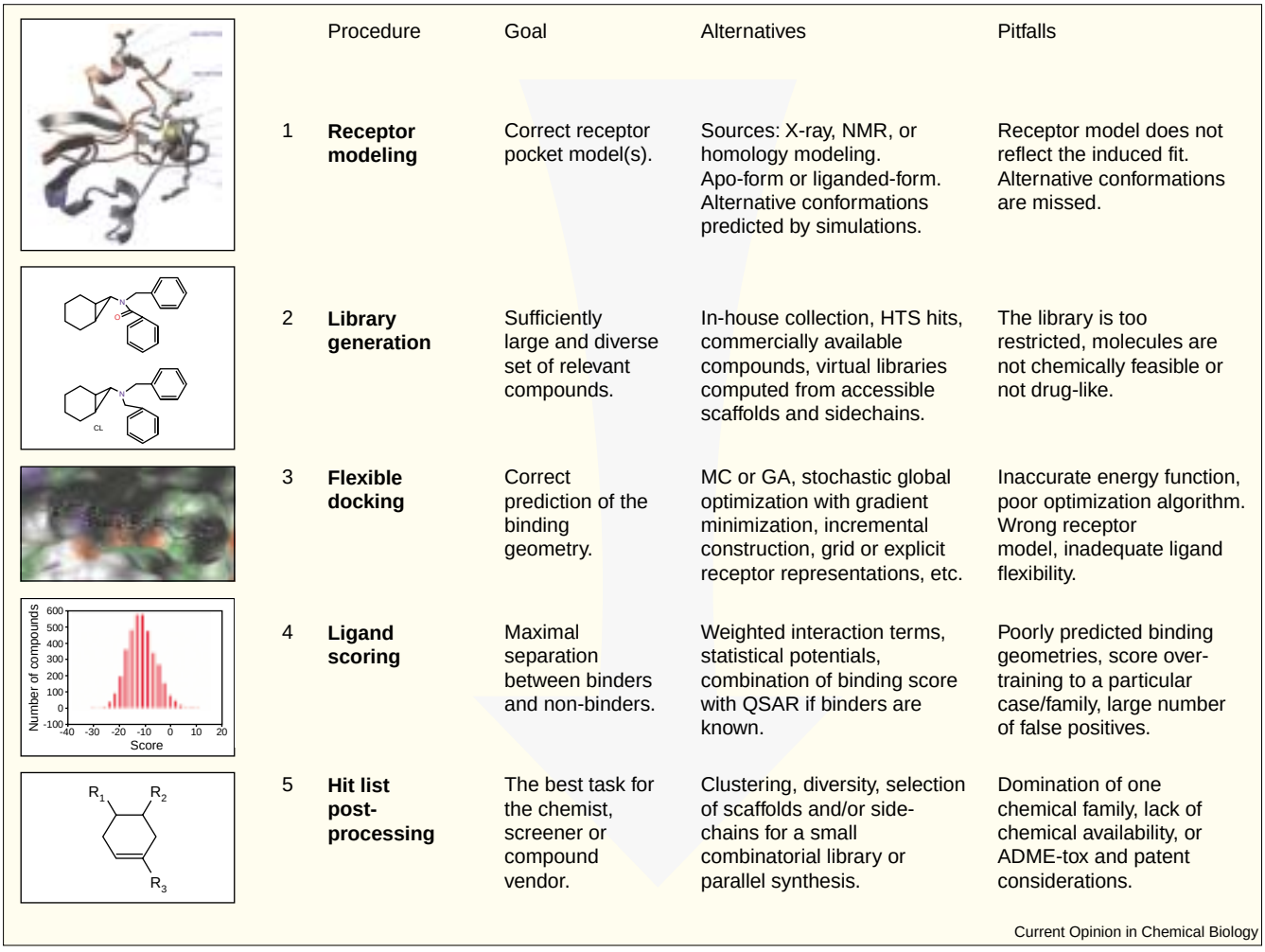
Flexible docking methods

Most of the existing established flexible docking algorithms, such as DOCK, ICM (Internal Coordinate Mechanics), FlexX, QXP, Ecepp/Prodock, Pro_LEADS, Hammerhead, FLOG, GOLD, LUDI, AutoDock and GREEN have been evolving for years. Most groups continue to improve their core docking procedures or develop additional protocols on top of them to answer a specific question.

FlexX is an incremental construction docking algorithm involving three steps: selection of base fragments of the ligand molecule, placement of base fragments in the active site, and incremental reconstruction of the whole ligand. In a recent paper from the FlexX group [7•] the performance of the algorithm was evaluated on a set of 200 complexes. An attempt was made to include explicit water molecules into docking simulations (‘Particle concept’ in [7•]). Possible water positions are pre-calculated, and during the incremental reconstruction stage water molecules are placed at the pre-computed positions if they can form additional hydrogen bonds to the ligand. In a test on 200 complexes with known structures, improvements were observed, but only for some targets (e.g. HIV protease complexes) containing a strongly bound water molecule. The overall effect of water inclusion, however, was marginal, with the number of complexes predicted to within 1 Å RMSD of experimental structure actually decreasing.

In contrast to the incremental construction algorithms, exemplified by DOCK and FlexX, the Internal Coordinate Mechanics (ICM) docking algorithm relies on global optimization of the entire flexible ligand in the receptor field. The ICM program [8] combines large-scale random moves of several types with gradient local minimization [9] and a history mechanism that both expels from the unwanted minima and promotes the discovery of new minima [10]. The random moves include pseudo-Brownian moves [11], optimally biased moves of groups of torsions [9] and single torsion changes. An extension and optimization of the energy terms and the algorithm for flexible grid docking was reported [12]. The optimized function included lone-pair-based hydrogen bonding, a smooth hydrophobic term, truncated van der Waals term and electrostatics. A benchmark of 51 complexes of 23 receptor proteins was used for validation and docking procedure optimization via a comprehensive cross docking of all ligands to all receptors. Of

Figure 1



Flow chart of the flexible docking and VLS procedure. ADME-TOX, adsorption, distribution, metabolism, excretion and toxicity; GA, genetic algorithm; MC, Monte Carlo.

the 51 complexes, 26 were predicted with better than 2 Å RMSD (~50%). The optimized set of parameters resulted in significant improvements of the docking predictions.

Trosset and Scheraga [13] extended the ECEPP3 core and the Monte Carlo Minimization algorithm and developed the program ProDock, in which the ligand is optimized by Monte Carlo minimization and the receptor is represented by grid potentials. The Bezier splines were used to calculate derivatives of the grid energy for efficient gradient minimization of a ligand.

New docking programs continue to emerge (e.g. MCDOCK [14], SEED (Solvation Energy for Exhaustive Docking) [15] DARWIN [16], MM (Mining Minima) [17], GLIDE (T Halgren, 'Rapid flexible docking of ligands to receptor sites with GLIDE', West Coast Annual Discovery 2001 Conference). Even more programs are in development. SEED [15] uses a large pre-set library of molecular fragments, which are docked into the binding site. The fragment–receptor interaction energy, which includes a sophisticated solvation term based on the generalized-Born approximation [18•], is subsequently evaluated for each fragment position, best-docked fragments are selected and merged using CCLD (Computational Combinatorial Ligand Design) generating a number of putative ligands. However, the validation of the approach was rather indirect. Because of the way docked molecules were constructed, it appears to be difficult to test the method on a large variety of complexes with known structures, limiting comparison with experimental structures to a few rigid molecules or to a qualitative similarity of generated and known ligands. A generalized-Born solvation model similar to the original SEED was re-implemented in DOCK [19•].

The MM algorithm for ligand docking was published by David, Luo and Gilson [17]. This optimizer generates a large number of conformations and evaluates them using

the CHARMM force field, keeps track of the minima sampled at every iteration, and recombines the best solutions similarly to a genetic algorithm. The search is guided away from the previously discovered minima in a manner similar to the ICM conformational stack mechanism [10] or Tabu Search [20,21]. The method was compared with AutoDock, FlexX and MCDOCK.

MCDOCK uses Monte-Carlo simulated annealing to identify the global minimum. In contrast to the ProDock and ICM algorithms, MCDOCK does not employ gradient optimization of the ligand. The conformations are generated with geometrical docking followed by energy-based docking. MCDOCK was successfully tested on 19 ligand–receptor complexes [14].

DARWIN — a new docking program based on a genetic algorithm and the CHARMM force field — was developed and tested on two carbohydrate-binding proteins [16]. The paper provides an insight into the importance of solvation effects. Initially, several water molecules observed crystallographically in the binding pocket were retained, and good docking results were obtained without any specific solvation term. However, when the waters were removed, no solution within 3 Å of experimental structure could be found. The results improved if the electrostatic term was completely omitted. Finally, when an electrostatic solvation term using the finite-difference solution of the Poisson equation was employed, the correct solution (RMSD less than 0.5 Å) was recovered. Encouragingly, the implicit solvent model could successfully substitute for the explicit water molecules, at least for the system tested.

Information about a bound ligand can facilitate docking of similar ligands binding in a similar mode. Fradera *et al.* [22] developed an extension to the DOCK4.0 program to incorporate the similarity of the docked molecules to ligands with known binding mode. The similarity can both guide the ligand during the docking process and modify the final binding score of a ligand. The method was applied to thrombin ligands.

A general comparison between different classes of global optimization algorithms for docking is difficult because of the critical dependence of the performance on details of the implementation. Diller and Verlinde [23] compared some stochastic methods with the incremental construction methods and concluded that stochastic methods have inferior performance.

To conclude this section we should say that, in a realistic case, an average reputable docking algorithm would dock only about 30–50% of the binders to the receptor pocket with RMSD less than 2–3 Å in about 1–3 minutes per molecule on a single processor. Of course, additional information, a favorable target or a selection of ligands may improve the results. Clearly, there is significant room for improvement that might be achieved through better force fields, and more rigorous optimization procedures.

Integrating docking and chemistry

In a straightforward application of VLS, molecules of interest are built in advance and were docked to a receptor pocket and evaluated. Instead of generating a large number of combinations in advance, however, the molecule can be 'built' into a pocket when needed. A large number of programs were designed to grow ligands atom-by-atom (Genstar, Legend, MCDNLG, CONCEPTS [24–27]), or fragment-by-fragment (Grow, LUDI, GrowMol, GroupBuild, SPROUT, BUILDER, SMOG and CONCERTS [28–36]). These methods struggle with two main problems: the low accuracy of docking and the force field, as well as a too-restricted or too-liberal assessment of chemical accessibility.

An approach based on the DOCK docking algorithm was reported recently [37]. DOCK was one of the first ligand-docking programs [38]. The initial version used rigid ligands; later, flexibility via incremental construction of the ligand in the binding pocket was incorporated. A recent paper by Makino *et al.* [37] describes how six types of reactions, utilizing combinatorial chemistry on a solid support, are used in combination with the DOCK conformational search to construct a ligand.

Usually, ligand-building algorithms attempt to design an ideal ligand directly in the active site. In practice, however, a single predicted ligand is uninteresting for a chemist because of low prediction accuracy (both false positives and false negatives), unexpected absorption, distribution, metabolism, excretion and toxicity properties, cross-reactivity, chemical accessibility, etc. Typically, the chemist can synthesize a small combinatorial set of compounds almost as easily as a single compound. Therefore, the task the chemist is confronted with is the selection of several good scaffolds for a particular target, or a family of targets, as well as the selection of the best sidechains for a given scaffold. These problems have been addressed in a recent paper by Lamb *et al.* [39], which enhanced the previously published CombiDOCK approach from the same group [40] from the comparison of sidechains to the comparison of three scaffolds docked to three related protein targets. The results are encouraging.

Chemical clustering of a hit list and retaining only the best representatives of each family can increase chemical diversity of the generated hits. This approach was successfully applied by the Shoichet group to thymidylate synthase, dihydrofolate reductase, and a lysozyme mutant [41].

Receptor flexibility

One of the biggest challenges of ligand docking is taking the flexibility of the protein binding sites into consideration (reviewed in [42]). There are five levels of sophistication in the consideration of receptor flexibility [43•,44]:

1. Use a static crystal structure of a receptor complexed with another ligand, or use an unliganded structure, the first is usually preferable.

2. Build a receptor model that tolerates ligand binding with some clashes without explicit repacking of the receptor sidechains (the model is static but permissive and implies multiple binding modes).

3. Use several alternative receptor binding site conformations for docking and merge the docking results.

4. Include partial receptor flexibility by allowing receptor relaxation for different trial conformations of the ligand.

5. Perform joint global optimization of molecular dynamics simulations of ligand and the receptor binding site.

The main problem with explicit inclusion of receptor flexibility into docking calculations is that it makes the results worse, not better. Also, the explicit treatment of receptor flexibility is too computationally expensive to include in VLS of large libraries. Therefore, most of the docking algorithms use levels (1) and (2) because ligand binding pockets are usually relatively rigid. Levels (4) and (5) are used for protein–protein docking [45] in which flexible sidechains at the interacting surfaces are unavoidable, and it has been demonstrated that the refinement of the docking solutions by global optimization of the surface sidechains does improve the results.

Broughton [44] successfully used short runs of molecular dynamics for incorporation of protein flexibility in protein–ligand docking that actually improved the results. This was achieved by a combination of statistical analysis from molecular dynamics runs with grid ligand docking. The method was applied to rank a homologous series of COX-2 ligands.

Schapira *et al.* [43•] performed explicit receptor sidechain and ligand co-optimizations on a small set of known ligands (level 5). An alternative receptor sidechain arrangement was identified in this simulation and the new receptor conformation was used in flexible ligand docking of a much larger set of compounds (level 3). Docking against several alternative static conformations and the explicit global optimization of a small set of ligands to flexible receptors may become a more popular approach, especially in cases where a set of ligands requiring the alternative packing is known.

Scoring

Let us make several general comments about scoring ligands. First, if a ligand is not docked correctly (see the docking section), there is no hope of calculating a correct score based on this docked conformation. Second, the scoring function of choice will strongly depend on its intended use. The best scoring function for ranking a large diverse virtual library (i.e. discriminate a small number of binders from hundreds of thousands of non-binders) is usually different from the scoring function optimized to explain binding of a small focused library of related compounds. Third, QSAR (quantitative structure/activity relationship) approaches, having very little to do with docking, can be used if the binding of a set of ligands is characterized experimentally. Most *ab initio* scoring functions, therefore, are based on interaction terms (e.g. DOCK force field or chemical scoring), whilst the scores can be further adjusted using QSAR analysis to the experimental binding affinity data (LUDI, ChemScore, SCORE).

The 'Fresno' scoring function [46] exemplifies the derivation of a scoring scheme for a particular receptor (MHC class I) based on two training sets and three-dimensional models of bound peptides. The authors achieved good prediction accuracy (around 1 kcal/mole) and described a method to re-optimize their scheme for a different class of receptors.

It has been shown repeatedly that most scoring functions fail to show significant correlations with binding constants when confronted with novel ligand–receptor systems, even though they are generally tuned well to predict binding constants for a training set. Because the primary goal of the screening score is to discriminate binding ligands from the background, it may be beneficial to optimize the scoring explicitly for the best differentiation between active and inactive ligands, rather than for the correct ranking of the binders.

A benchmark set of 23 diverse receptors and 63 ligands was used to tune the scoring function for the best separation of binders and non-binders across the set [47•]. The discrimination function is calculated from the scores derived as a weighted sum of five physical terms (hydrophobicity, solvation electrostatics, hydrogen bonding, ligand deformation energy and the van der Waals ligand–receptor interaction energy). This function was recently tested by Brooks and colleagues (personal communication) and in a comparison of screening algorithms and showed a strong selectivity.

An alternative to an empirical function based on predicted physical interaction terms is a 'knowledge-based' function that uses statistics for the observed inter-atomic contact frequencies and/or distances. These methods assume that derived statistical preferences implicitly reflect favorable/unfavorable interactions between functional groups (see DrugScore [48], PMF [Potential of Mean Force] [49] BLEEP [50]).

Combining multiple scoring functions may reduce the number of false positives, which are likely to be different in different scoring schemes. This will work, however, only if a substantial fraction of the database (~10%) is retained for each score to avoid false negatives. A study covering 13 scoring functions and two docking methods (DOCK and GAMBLER, an in-house method at Vertex) [51•] found that consensus scoring may dramatically reduce the number of

false positives identified by individual scoring functions. Three target receptors (p38 MAP kinase, inosine monophosphate dehydrogenase and HIV protease) and several hundreds of active ligands plus 10,000 random compounds were used as a benchmark. Similar results were reported by Bissantz *et al.* [52•] (see below) who concluded that consensus scoring improved the hit rate several-fold.

Comparisons of docking and scoring methods

How often is a docking geometry prediction correct and what is the geometrical accuracy of such a prediction? The majority of the authors consider predictions within a 2 Å RMSD from the X-ray structure satisfactory. Even though this error seems high (certainly enough to break a hydrogen bond), the essential chemical groups of a ligand at 2 Å RMSD are usually placed correctly. Most of the deviation comes from weakly interacting groups at the periphery. Larger molecules often retain essential features of the interactions even at 3 Å RMSD values.

Usually, authors test their methods by re-docking ligands to the crystal structure of their receptor. However, the benchmarks vary in the number of ligand–receptor pairs, their selection, and the details of the set-up. Some authors do not regenerate the conformations of the ligands from chemical structures, or energy-minimize complexed receptor structures before using the protein in the docking simulation, which may make reconstruction of the complex easier. These differences make it difficult to compare various methods without actually testing them side-by-side, on a consistent benchmark. This need was recently addressed in a number of publications described below.

DOCK and FlexX were compared in a study based on a set of 32 thrombin inhibitors representing different chemical classes of compounds [53]. Public and proprietary X-ray complexed structures were used to evaluate the simulation results. DOCK4.0 with chemical scoring was found to be superior to DOCK4.0 with energy scoring and FlexX with the Böhm scoring function. The performance of all algorithms was relatively poor, however, with only 10–35% of the test compounds docking correctly to within 2 Å RMSD of the experimental structure. This is in some contrast to the 46.5% of better than 2 Å solutions reported for FlexX by its authors [54] on a set of 200 protein–ligand complexes.

Another comparison of DOCK and FlexX has been reported [55]. In this study, 61 inhibitors of matrix metalloproteinase 3 (MMP3) were docked, the resulting conformations compared with those in X-ray structures, and several scoring functions tested for correlation with the experimentally determined binding constants. It was found that DOCK4.0 with PMF performed best in the prediction of the bound conformation (mean RMSD 1.8 Å), only four compounds had the wrong binding mode (RMSD >4 Å), followed by FlexX (2 Å and 12 respectively) and DOCK4.0 with force field score (2.5 Å and 9 respectively). However, FlexX had significantly better RMSD for the compounds with correctly predicted binding mode.

Bissantz *et al.* [52] compared three docking methods (DOCK, FlexX and GOLD) and seven scoring functions (CHEMSCORE, DOCK, FlexX, FRESNO, GOLD, PMF, SCORE). The benchmark set consisted of 990 random compounds from ACD (Available Chemicals Directory) and 10 known ligands for each of the two receptors estrogen receptor (ER) and thymidine kinase (TK). GOLD came out as a better docking tool with 6 out of 10 TK ligands within 2 Å RMSD (4 and 3 for FlexX and DOCK, respectively), and similar results for the easier ER test. The study further shows that the performance of the scoring functions is often inconsistent across different systems, with DOCK scores performing well for the apolar binding site of ER and poorly for the TK, whereas the FlexX score behaved in the opposite manner.

Affinity prediction methods were tested on a set of 30 glycogen phosphorylase (GP) inhibitors. X-ray structures of complexes were available for all ligands, no docking was performed. Five methods based on QSAR (one 2D and four 3D) performed predictably well, because the compounds in the set were closely related and were used themselves to derive the model. Two receptor-based functions, LUDI and SBEP (an empirical function constructed from four physical terms with weights adjusted by regression analysis or using neural network to fit the experimental data) performed poorly. The LUDI function showed no significant correlation with observed binding affinity [56].

Applications of VLS to lead discovery

It is already common knowledge that many leads are optimized through cycles of structure-based drug design after HTS. However, more and more lead candidates are now being discovered directly on the basis of VLS results.

New compounds can be identified even if the relevant crystal structure was not available and a model was successfully used instead. Several new antagonists of human retinoic acid receptor-alpha were discovered with ICM virtual screening of ACD to a model rebuilt from an agonist-bound retinoic acid receptor-alpha structure [43•]. The idea for repacking of helix 12 and the connecting loop came from the antagonist-bound estrogen receptor-alpha. Out of 30 experimentally tested compounds three were confirmed as antagonists [43•].

The ICM flexible docking procedure was also applied to dock a subset of ACD and find specific binders of the RNA hairpin HIV-1 TAR RNA [57]. The scoring function based on the physical terms was trained on several RNA–ligand complexes. Eight of the highest-ranking compounds selected by the procedure were assayed for inhibition of the Tat–TAR interaction, and two exhibited a CD50 of ca. 1 μM.

ACD was screened for the inhibitors of kinesin using DOCK [58]. A DOCK screen of ACD also yielded novel inhibitors of thymidilate synthase [59]. In yet another DOCK-based study, the screening of a small virtual library was performed to discover inhibitors of the hypoxanthine-guanine-xanthine phosphoribosyl transferase [60].

Using SANDOCK, ACD and the Cambridge Crystallographic Database were screened against the FK506-binding protein [61]. The program EUDOCK was used to search a subset of ACD for farnesyltransferase inhibitors [62].

The design of potent non-peptide thrombin inhibitors using LUDI has been reported [63]. The best compound found in this study had a K_i of 95 nM. The X-ray structure of the complex was determined. The predicted conformation was found to be in reasonable agreement with the experimental result. Although the high affinity of the best ligand found is remarkable, this study should be considered as an example of lead optimization rather then discovery, because the explored chemical structures were largely limited to the derivatives of the known inhibitor (benzamidine). LUDI was also applied to discover novel inhibitors of DNA gyrase [64].

Conclusions

Ab initio docking of a diverse set of drug-like compounds to a receptor can be performed in about 1 to 5 minutes per compound per processor with 10 to 50% docked with less than 2 Å RMSD from the correct solution. The main challenge in the discovery of the lead candidates is the discrimination between a small number of binders and a very large number of non-binders. Better force fields and better accounts of the receptor and ligand flexibility are critical, whilst global optimization methods of different types demonstrate comparable performance. Experimental information about binders to a given receptor can be used to improve the efficiency and accuracy of the docking and scoring algorithms. Awareness of the requirements and practical preferences of the chemist need to be better understood and addressed. Virtual ligand docking and screening can be applied to selections of individual chemically accessible lead candidates, selection of the sidechains for a given scaffold, and/or selection of the scaffolds. The number of success stories from VLS is growing quickly.

Update

FlexE is an extension of the FlexX docking algorithm that can take into account the receptor flexibility using a predefined ensemble of receptor structures instead of a single rigid one [65]. The ensemble is derived from multiple X-ray structures or by homology modeling, but can potentially be generated by Monte Carlo or molecular dynamics simulation. On a test set, results similar to sequential docking to all alternative receptor conformations were obtained, with significantly (~twofold) lower run times. Chen and Zhi [66] report a novel application of high-throughput docking that they call 'inverse docking'. A database of binding sites was created from Protein Data Bank receptor structures (2700 entries), and screened against specific small-molecule ligands. Test screens for tamoxifen and vitamin E show some meaningful hits. Diller and Merz [67] report a fast docking algorithm for library design. The method combines combinatorial interaction 'hot spot' matching for the generation of initial docked conformation with subsequent refinement of solutions through minimization of a crude interaction potential. A test case of library design shows modest (1.7- to 3.3-fold) enrichment of the (simulated) designed library. Schafferhans and Klebe [68] developed a new approach, DragHome, which combines 3D QSAR methodology using known ligands with structural information about the binding site obtained from homology modeling of the receptor. Multiple models or conformations of the receptor can be utilized through averaging of the binding-site representation. The method was tested on thrombin homology models at 40% and 28% identity.

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