

# Docking: Successes and Challenges

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**Abstract:** The state of the art of various computational aspects of docking-based virtual screening of database of small molecules is presented. The review encompasses the different search algorithms and the scoring functions used in docking methods and their applications to protein and nucleic acid drug targets. Recent progress made in the development and application of methods to include target flexibility are summarized. The fundamental issues and challenges involved in comparing various docking methods are discussed. Limitations of current technologies as well as future prospects are presented.

## 1. INTRODUCTION

Molecular recognition plays a key role in promoting fundamental biomolecular events such as enzyme-substrate, drug-protein and drug-nucleic acid interactions. Detailed understanding of the general principles that govern the nature of the interactions (van der Waals, hydrogen bonding, electrostatic) between the ligands and their protein or nucleic acid targets may provide a conceptual framework for designing the desired potency and specificity of potential drug leads for a given therapeutic target. Practical application of this knowledge requires structural data for the target of interest and a procedure for evaluating candidate ligands. To this end, a variety of computational docking methods are available [1-5]. These provide one approach to the ranking of potential ligands with respect to their ability to interact with a given target.

Computational docking of a small molecule to a biological target involves efficient sampling of possible poses of the former in the specified binding pocket of the latter in order to identify the optimal binding geometry, as measured by a user-defined fitness or score function. X-ray crystallography and NMR spectroscopy continue to be the primary source of 3-dimensional structural data for protein and nucleic acid targets. In favorable cases where proteins of unknown structure have high sequence homology to known structures, homology modeling can provide a viable alternative by generating a suitable starting point for 'in silico' discovery of high affinity ligands. Databases of drug-like molecules such as MDDR [6] or CMC [7], as well as other small molecule databases including ACD [8], CSD [9] and NCI [10] are available. In addition, very large virtual libraries can be enumerated from building blocks or fragments and known chemical reaction schemes.

During computational docking, a pose is typically generated, scored and compared to the previous pose(s). The

current pose is then accepted or rejected on the basis of the score for that pose. A new pose is then generated, and the search process iterates to an endpoint. Thus, searching and scoring can be tightly coupled in docking. Reliable rank ordering of the ligands based on their docked scores such that the scores correlate with experimental binding affinities appears to be even more challenging than searching the conformation and orientation space [11-13]. A recent trend has been to employ consensus scoring (apply a number of score functions to the same docked pose identified by docking) to eliminate false positives [13, 14].

The utility of virtually screening a large database of molecules depends on the ability to provide a hit rate much better than random selection. 'In silico' approaches need to be robust and fast in order to have a major impact on lead identification. The standard test that has emerged for docking-based virtual screening protocols evaluates the ability of the docking method to prioritize known active molecules from a database comprised largely of molecules known or presumed to be inactive. While many docking tools have shown utility in this context, comprehensive method comparisons have not been reported.

Over the last few years a vast amount of effort has been directed toward developing efficient docking methods and scoring functions as tools for the identification of lead compounds. Considerable progress has been made in the computational prediction of ligand-target binding modes. A number of review articles in this emerging area of research have been recently published [15-19]. While not exhaustive, this review highlights current computational docking technology in the context of its successes, failures, limitations, challenges and future prospects.

## 2. DOCKING METHODS

The complexity of computational docking increases in the following order: (a) rigid body docking, where both the receptor and small molecule are treated as rigid. (b) flexible ligand docking, where the receptor is held rigid, but the ligand is treated as flexible; and (c) flexible docking, where

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both receptor and ligand flexibility is considered. Thus far, the most commonly used docking algorithms use the rigid receptor/flexible ligand model.

The principal docking methods that are used extensively employ search algorithms based on Monte Carlo, genetic algorithm, fragment-based and molecular dynamics. Some programs that are well-suited for high throughput docking of a large database of molecules include: DOCK [1, 2], FlexX [3], GOLD [4], and ICM [5]. The search methods and score functions of various docking programs as well as the test cases used in validation studies have been described in a recent review [15]. It is of interest to compare different docking programs and rank their relative performance. Unfortunately, such attempts have proven to be extremely difficult for several reasons. Most investigators have access to only a limited number of methods for evaluation. The test cases tend to be limited in the number and type of targets evaluated. Finally, each algorithm combines a particular search strategy and a particular scoring function. Although one would like to evaluate these two separately, in general one cannot change the scoring function that drives the docking. Retrospective scoring, while useful, does not address how the primary score function affects the efficiency and accuracy of a given search algorithm.

The fundamental issues and challenges involved in comparing docking-based methods for virtual screening of a database of molecules are discussed in detail in a later section.

### 3. STRUCTURAL DATA

#### 3.1. Ligand Representations

The question of appropriate representation of molecules in databases has been addressed recently<sup>§</sup>. For most docking programs, the protomeric and tautomeric states of the small molecules to be docked are user-defined. Typically, the structure most likely to be dominant at neutral pH is generated. The structures can be further adjusted by adding or removing hydrogens provided approximate pKa values are known *a priori*. It is extremely important to make sure that 'accurate' atom typing occurs. The wrong definition of donor and acceptor properties of heteroatoms may lead to serious docking errors. For example, Watson and Crick originally assigned the wrong tautomeric formulae (enol forms) for the nucleic acid bases and thus could not build a helical model with purine-pyrimidine hydrogen bonded base pairs. However, once the correct tautomeric structures (keto forms) of the bases were assigned, all the key features of the three-dimensional structure of doublehelical DNA were readily accounted for [20]. In cases where the stereochemistry of a synthesized compound is unknown or ambiguous, it would be beneficial to generate all possible diastereoisomers and dock them individually to the receptor. Commercial software programs for the enumeration of all possible diastereoisomers of a given compound include: Stergen [21], Stereoplex [22] and PipelinePilot [23].

#### 3.2. Receptor Representation

##### 3.2.1. Receptor Crystal Structures

The quality of the receptor structure employed plays a central role in determining the success of docking calculations [24–26]. In general, the higher the resolution of the employed crystal structure, the better the observed docking results. Schapira *et al.* using ICM for docking showed that they could reproduce the known binding modes of ligands to within 1 Å of the bound conformations, in cases where the resolution of the employed co-crystal structures were better than 2.0 Å [26]. In addition, it is important to insure that the B-factors of the atoms in the binding site region are reasonable, as high values imply that their coordinates are less reliable. A recent review of the accuracy, limitations and pitfalls of the structure refinement protocols of protein-ligand complexes in general provided a critical assessment of the available structures [27]. The importance of the pH dependence of ligand binding modes was highlighted. Uncertainties in locating the ligands ('mistaken identity') in the co-crystal structures as well as the subjective nature of deriving good quality protein models were emphasized in the context of published structures. The reliability of the ligand structures found in co-complexes has been questioned also. Even at high resolution, the difficulties in defining ligand atomic positions unambiguously can be attributed to the disparity between the high-quality dictionaries of bond lengths, bond angles and torsions available for proteins and nucleic acids structure refinement and those available for small organic molecules [28, 29]. Regardless of the possible ambiguities, success has been reported for numerous high throughput docking studies using X-ray receptor structures. Recent examples of this type of study include: kinesin [30], thymidylate synthase [31], phosphoribosyl transferase [32], farnesyltransferase [33], FKBP12 [34], HIV protease [35], beta-lactamase [36], and PTP1B [37].

##### 3.2.2. Receptor Homology Models

In the absence of an experimentally determined three-dimensional structure of a target of interest, homology models have been used for the purpose of docking small molecules. Varying degrees of success have been reported on docking small molecules to receptor homology models. Accurate homology models can be built provided that the sequence identity of a given target sequence is > 50% to a known structure template [38]. However, homology models based on a wide range of sequence identity [30–70%] to template structures have been reported [39]. A number of programs are available for building homology models [40–43]. Modest homology model building efforts could potentially create receptor structures for entire target families, e.g. nuclear hormone receptors (NHR's), G-protein coupled receptors (GPCR's) and kinases. In addition, homology modeling is a relatively inexpensive method for generating a variety of receptor conformations using either a single template or multiple template structures. The recently proposed inverse docking method [44] could be applied to identify the correct target protein structure (from a family of receptor structures) that specifically binds a given small molecule, thereby enhancing the current understanding of selectivity.

<sup>§</sup> Pearlman RS. COMP-232, 224th ACS National meeting: Boston, USA, 2002.

Klebe's group has developed the novel method [45], DRAGHOME, to dock ligands to receptor homology models. In this approach the quality of the homology model is improved by including information derived from known ligand structure-activity relationships (by aligning ligands relative to each other and relative to the protein model). Protein structure modeling and ligand data analysis are repeated in cycles to arrive at a self-consistent model.

The abundance of X-ray structures as well as the common fold found in kinases may account for the relative success reported in docking to kinase homology models compared to other target families. Based on high throughput docking, potent and selective chemo-types for a few kinases have been successfully identified. A homology model of the protein kinase CK2 was employed in the docking of 400,000 compounds (using DOCK) on a grid computing system [46] by means of a SETI@home-like technology, over PC networks. This study resulted in the discovery of a potent inhibitor ( $IC_{50} = 80$  nM) with high selectivity [47]. Diller and Li [39] have assessed the value of high throughput docking to homology modeled kinase structures (tyrosine and serine/threonine kinase families). In this study a conformational database of 32,000 compounds seeded with known inhibitors was generated using Catalyst [48], and the conformers were then docked using the program LibDock [49]. Known inhibitors were enriched by a factor of 4 versus what would be expected with random selection when the top 5% of compounds were examined. The relative performance of this docking procedure for each of the six kinases studied has been analyzed in detail. Due to the general propensity of the kinases to form more closed structures around the ATP binding pocket as well as the induced fit phenomenon observed upon ligand binding in a number of cases, the authors recommend using 'open' structures as templates for building homology models.

In another study, potent CDK4 inhibitors were identified using a CDK2-based homology model [50]. New scaffolds that satisfy receptor structural requirements were identified using LEGEND [51]. However, most of the compounds were neither commercially available nor synthetically feasible. Subsequently, SEEDS [50] was used to pick out a core substructure and form queries to search the ACD. Application of filters to discard undesirable functional groups retained 382 compounds that were purchased and screened. 12 hits were identified with low activity ( $IC_{50} = 16-450$   $\mu$ M) that were found to cluster into 4 distinct chemotypes. Follow-up rounds of synthetic chemistry ultimately yielded the diarylurea inhibitor (42 nM). The predicted binding pose of this potent compound is in good agreement with the subsequently determined co-crystal structure.

Schapira *et al.* [26] have reported the successful validation of virtual screening with a set of 19 NHR structures (18 crystal structures and one homology model). A diverse set of 5000 compounds including 78 known ligands for the 19 NHR's in the study was docked using ICM. The results at the top 1% level indicate that the enrichment factors ranged from 33 to 100 for all but one of the targets, implying significant differences in docking efficiency from one receptor to another. In 11 out of 16 structures for which ligand-bound complexes were available the known binding

modes were reproduced to within 1  $\text{\AA}$ . In the case of the homology modeled glucocorticoid receptor, all known ligands were found in the top 1%. A number of specific inhibitors for other nuclear hormone receptors were also well-ranked in the docking with this receptor, illustrating the difficulties in achieving ligand discrimination. In another study [52], 250,000 compounds were docked using ICM to a homology model of antagonist-bound thyroid hormone receptor. The top-scoring 1000 molecules were further subjected to a refinement protocol. Based on the final selection, 100 compounds were retained for *in vitro* study. 14 antagonists were identified with  $IC_{50}$  ranging from 1.5 to 30  $\mu$ M. Nine of these ranked in the top 2.4%.

Bissantz *et al.* docked a database of compounds to homology models of GPCRs with the aim of discovering agonists and antagonists [53]. They used three different docking programs (DOCK, FlexX and GOLD) and seven different secondary scoring functions. A database of 1000 compounds was generated by randomly choosing 990 compounds from from a version of the ACD that was filtered to exclude compounds with reactive functionality, inorganic molecules and molecules with molecular weight less than 250 or greater than 600 and seeding in 10 known ligand structures. Antagonist screening was relatively straightforward, while agonist screening required extensive binding site adjustment in the presence of modeled binding modes for several known ligands. In the most promising cases of antagonist screening, hit rates 20- to 40- fold higher than those expected from random selection were observed.

### 3.2.3. Effect of Receptor Structure on Docking Results

The relative performance of docking calculations with respect to receptor structure quality has been addressed by McGovern and Shoichet [25]. They performed exhaustive docking screens of a database of 95,000 compounds against 10 different receptors for which ligand-bound, apo and homology modeled structures were available. Docking performance was assessed by the observed enrichment of known ligands, as defined by their therapeutic target listed in the MDDR, among the top scoring hits. The best overall enrichment was produced by the ligand-bound structure in seven systems, the apo structure in two systems, and the homology modeled structure in one system.

## 4. RECEPTOR FLEXIBILITY

It is well known that macromolecules often undergo conformational change, or induced fit, upon ligand binding in order to maximize energetically favorable interactions with the ligand or solvent [54, 55]. The driving force behind most induced fit mechanisms is hydrophobic interactions or hydrophobic collapse of the receptor around the bound ligand [56]. There are varying degrees of receptor flexibility. Conformational flexibility does not necessarily need to involve domain, tertiary, and/or secondary structure motions but may consist solely of subtle side-chain adjustments. In a protein-protein docking study, Cummings *et al.* [57] showed that truncation of a single Arg side-chain facilitated reconstruction of diubiquitin from two copies of the uncomplexed monomer. One comprehensive study indicates that in ~85% of (a total of 3,287) cases three or fewer active site residues undergo conformational change upon ligand binding [58].

Optimal ways of using multiple protein structures are currently being explored in order to treat protein flexibility in a more realistic manner [59-61]. It has been noted that the successes and failures of docking simulations have been explained on the basis of thermodynamic properties determined from equilibrium simulations and the shape of the underlying binding energy landscape, funnel-like for rigid docking and rugged for flexible docking [62].

An obvious caveat with most docking approaches is the rigid receptor hypothesis [63, 64]. The major drawback of the rigid receptor docking approach is that it may lead to incorrect ligand binding modes or poor docking scores, thus overlooking prospective drug leads. This, coupled with the fact that conformational changes within the receptor may have important implications with respect to ligand selectivity, illustrates the importance of incorporating receptor flexibility in computational drug design. Significant progress has been achieved in the last few years in addressing this critical issue. The degree of flexibility one could incorporate in a given experiment is directly proportional to computational complexity and cost. A few of the more elegant methods simulating receptor flexibility are described below.

#### 4.1. Soft Docking

Soft docking algorithms attempt to allow for flexibility of the receptor and ligand structures by using a relaxed representation of the molecular surface. An efficient scheme to handle receptor flexibility in an implicit fashion is to use additional energy terms (usually van der Waals) in the empirical scoring function. From a computational point of view it is advantageous to treat flexibility using the above approach, since the docking engine remains the same as in rigid receptor docking.

The soft docking concept, originally proposed by Jiang and Kim, describes the molecular surface and volume as a "cube representation" [65]. This cube representation implies implicit conformational changes by way of size/shape complementarity, close packing and, most importantly, liberal steric overlap. In recent years the soft docking concept has evolved primarily toward use in protein-protein docking [66-70] and protein-receptor modeling combined with experimental NMR data [71-73].

#### 4.2. Side-Chain Flexibility

Allowing active site side-chain flexibility is another way to provide receptor flexibility. One method, originally proposed by Leach [74], uses pre-generated side-chain rotamer libraries that subsequently are subjected to optimization during a ligand docking procedure via the dead-end elimination algorithm. The optimized ligand/side-chain orientations are then scored in order to rank the lowest energy combination of side-chain and ligand conformers [75].

Gilson has recently enhanced the Mining Minima optimizer [76, 77] by incorporating side-chain flexibility [78]. The algorithm allows conformations of user-selected side-chains in the active site to be optimized along with the conformation and position of the ligand. This is accomplished by computing energies associated with the selected side-chains as if they belonged to the ligand.

Another docking procedure has automated the 'user-defined' selection of active-site, flexible residues by way of the SOFTSPOTS algorithm [79]. SOFTSPOTS makes use of a knowledge-based function that identifies active site residues most likely to undergo conformational change upon ligand binding. Usually only a few hydrophobic residues are selected, depending on location relative to ligand position and secondary structure assignment. The accompanying PLASTIC algorithm [79] generates the side-chain rotamers, or a minimal conformational manifold, prior to docking calculations.

Molecular dynamics and Monte Carlo simulations have also been used to explicitly model side-chain movement. Nakajima's method introduces multiconformational molecular dynamics simulations to broaden the range of side-chain conformers sampled and in effect smoothening the energy surface so that barriers can be overcome [80].

#### 4.3. Experimental and Theoretical Ensemble Docking

Ensemble docking has gained considerable attention as a method of incorporating protein flexibility in computational drug design. In most cases the full receptor ensembles are generated by molecular dynamics, Monte Carlo simulation or homology modeling methods. The ensembles can be generated experimentally - from NMR solution structure determination, or, in a few cases, multiple x-ray crystal structures. Comparisons have shown that there is significant overlap of dynamic information content between theoretically derived molecular dynamics ensembles and experimentally derived NMR ensembles [81]. However, in the case of RNase H1, NMR ensembles span more conformational space for both backbone and side-chains than a 1.7ns molecular dynamics simulation of the same protein [82].

A nice example of docking with experimentally derived ensembles is presented by Knegtel and coworkers [83]. DOCK was used with ensembles of both NMR and crystal structures to yield a composite single scoring grid, based on energy-weighted and geometry-weighted averaging methods. The single scoring grid method outperforms most of the grids derived from individual structures of an ensemble, and in the case of NMR generated ensembles, the minimized average structure.

A united protein description based on a superimposition of ensemble structures, either theoretically or experimentally generated, is the basis of the FlexE algorithm. FlexE treats both receptor flexibility using external ensembles and ligand flexibility, using the incremental construction algorithm. These are then recombined in a combinatorial fashion. By treating the system this way the interactions are not biased by averaging over distinct alternative instances, which may show unrealistic protein conformations [84].

Various techniques have incorporated molecular dynamics as the source of ensembles for docking. Two recent papers detailing improved prediction of binding energies by the use of short-run molecular dynamics have appeared in the literature. Ensembles from <100 ps molecular dynamics simulations improved known ligand enrichment with dihydrofolate reductase, cyclooxygenase-2 [85] and HIV-1 protease [86]. These results indicate that small structural

changes can greatly influence ligand scoring. Low-mode docking methodology has been used to take advantage of low-frequency atomic vibrations as induced-fit modeling [87, 88]. Two HIV integrase inhibitors were docked into the apo form of HIV integrase, followed by minimization; Low Mode Docking (LMOD) searches were then carried out to find low-energy binding orientations. Low energy complexes (2 kcal/mol above the global minimum) were found with a Root Mean Squared Deviation (RMSD) of 0.392 Å from the experimental binding conformation.

Ensembles generated from 300 ps molecular dynamics simulations in explicit water were used as a thermally averaged set of conformers involved in receptor/ligand binding. The ensembles were then subject to Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) [89, 90] calculations to measure free energies of receptor ligand binding. The theoretically determined free energies of binding for nine avidin and streptavidin-ligands were highly correlated with experimentally determined free energies of binding [91]. Another molecular dynamics method, the "Relaxed Complex" method, has been proposed by McCammon's group [92]. This method takes advantage of inherent, fleeting (and often high energy) protein conformations that may bind ligand. These rare conformations are generated via long-run molecular dynamics simulations of unliganded receptor giving the final receptor ensembles a wide range of energy.

#### 4.4. Hybrid Techniques

It should be mentioned that hybrid techniques for incorporating receptor flexibility into docking experiments are beginning to appear in the literature. A combined method of soft docking and side-chain optimization has recently been reported for protein-protein docking [93]. This procedure, DISCO, combines the use of soft receptor potentials as defined with the ICM docking engine, and interface residue side-chain optimization. Another hybrid methodology refines the aforementioned "Relaxed Complex" approach to take advantage of MM/PBSA scoring [94]. The authors show that the average distribution of docking free energies range from 2 to 3 kcal/mol, which is sufficient to misrank a potent drug candidate as a weak binder (and vice versa). However, by combining MM/PBSA and the "Relaxed Complex" method, they are able to show that the best ranked binding modes agree with x-ray structures. FDS (Flexible ligand and receptor Docking with a continuum Solvent model) is another hybrid technique [95]. This method initially docks the ligand into the protein active site based on satisfying possible sets of hydrogen bonds using graph theory. The docked compounds are then filtered by cluster analysis to reduce the number of structures submitted to a modified Monte Carlo algorithm that uses a Generalized Born Surface Area (GBSA) solvent model [96]. Optimization with active site side-chain rotamer libraries is performed and the resulting complexes are treated with a softening function for non-bonded force field terms. The rigid docking scheme was able to reproduce known binding modes in 13 cases compared to 11 cases (out of a total of 15 test cases) for flexible docking. Interestingly, the majority of the 11 binding orientations were not uniquely identified but were found in clusters of

low energy structures, therefore supporting the presence of a rugged docking energy hypersurface.

## 5. PROTEIN-LIGAND BINDING CONSIDERATIONS

### 5.1. Specific Interactions that Influence Ligand Binding

Important forces in the interaction of small molecules with their macromolecular targets include hydrogen bonding, charge-charge interactions and hydrophobic interactions. It is critical that any force field or scoring scheme designed to correlate with experimental binding energies include the appropriate reference state and account for the change in entropy of the system upon ligand binding. This is a tall order. Early docking methods incorporated only a van der Waals-like term [2]. This quickly expanded to include electrostatic interactions, which account for hydrogen bonding interactions as well as explicit charge-charge interactions [97]. More recently, scoring schemes have included Poisson-Boltzmann [89] or Generalized Born [96] treatments to mimic solvent effects.

While each of these forces plays a role in the interaction of ligands with their macromolecular targets, the dominant forces are different for each ligand-target pair. For example, the trypsin-like serine proteases bind an arginine in their P1 pockets and having a charge in this pocket is important in the binding of a large majority of inhibitors of these proteases [98]. On the other hand, the substrate binding site of HIV1 protease is quite hydrophobic as are many of its inhibitors. Even though HIV1 protease binds a peptide and has hydrogen bonding partners for several amides, the potency of inhibitors is poorly correlated with number of hydrogen bonds [99]. This does not however mean that any particular hydrogen bond made by a particular inhibitor could be eliminated.

### 5.2. Solvent Effects

Due to the complexities involved in computing ligand binding free energies, current docking methods use indirect methods at best to account for solvent effects in the energy calculations. The free energy perturbation [100] and linear response approximation calculations [101] are the most rigorous and accurate methods to compute binding affinities, but are too slow to be applicable in docking-based screening. A number of simple approximations have to be invoked so as to capture the essence of solvent effects at a reasonable additional computational cost. Implicit solvent methods (continuum dielectric model) are routinely being implemented in docking calculations [102-104]. In particular, GBSA [96] and PBSA [89] methods have been successfully applied to compute free energies of receptor ligand binding. Improvements in the ranking of known ligands and better discrimination amongst the rest of the compounds in the database were achieved by correcting for ligand solvation energy [105]. Solvent effects may play an even larger role in the case of docking small molecules to nucleic acid targets, as compared to protein targets.

## 6. PRE-DOCKING COMPOUND FILTERING

Prior to carrying out docking calculations, it can be beneficial to pre-select the database of compounds to be

docked by applying hierarchical filters so as to produce a 'focused' database. Such filters often drastically reduce the number of compounds that need to be evaluated in the more demanding docking calculation. An illustration of such an approach has been recently reported in a lead discovery study involving human carbonic anhydrase II [106]. Starting from a set of 90,000 compounds, the successive application of 2-D substructure queries to identify known metal-binding groups, 3-D pharmacophore based queries, and flexible superposition reduced the database to 100 compounds. Docking calculations of the selected 100 compounds with FlexX identified four potent inhibitors with activities in the nanomolar range. Subsequent crystallographic studies confirmed the predicted docking poses of two of these hits [106]. In the general case, a suitable set of pharmacophores can be derived by performing a binding site analysis to identify regions of favorable protein-ligand interactions. An excellent review has recently been published describing the application of pharmacophore based modeling methods in discovering new leads in the absence of structural data [107]. A hybrid approach in which initial pharmacophore-based filtering was followed by subsequent docking of a small subset of compounds yielded novel inhibitors of alanine racemase [108] and *Plasmodium falciparum* dihydrofolate reductase (PfDHFR) [109].

## 7. SCORING

Scoring functions can be broadly classified into three distinct categories: knowledge-based, empirical and force-field-based. Knowledge-based scoring functions rely on statistical means to extract rules on preferred, and non-preferred, atom pair interactions from experimentally determined protein-ligand complexes. The rules are interpreted as pair-potentials that are subsequently used to score ligand binding poses. The PMF score [110], for example, is a well-known knowledge-based scoring function. Empirical scoring functions sum enthalpic and entropic interactions with the relative weights of the terms based on a training set of protein-ligand complexes. The weights are assigned by regression methods that are used to fit the experimentally determined affinities. The interaction terms often include Van der Waals, electrostatic interactions and hydrogen bonds. Examples of empirical scoring functions include PLP [111], ChemScore [112] and the FlexX [3] scoring function. Force field scoring functions are similar to empirical scoring functions in that they predict the binding free energy of a protein-ligand complex by adding up individual contributions from different types of interactions. However, they differ from empirical scoring functions in that the interaction terms are derived from physical chemical phenomena as opposed to experimental affinities. Examples of force field scoring functions in docking programs include the energy score in DOCK [97], the score function used for single ligand docking in DOCKVISION [113, 114] and that used in GOLD [4]. Several reviews on the use of various scoring functions employed in docking calculations have been published [11, 13, 16, 18, 115]. The diversity of currently available score functions for docking, and the fact that each docking program uses its own built-in scoring scheme, underscores the lack of a universal scoring function.

Wang *et al.*, [116] have recently performed an extensive comparative analysis of the performance of eleven different scoring functions on 100 protein-ligand complexes with respect to their abilities to reproduce experimental binding affinities as well as the observed binding modes. In an attempt to isolate the scoring problem from the search problem, and in sharp contrast to other published studies (where *only* the best docked poses from a given docking program were re-scored using a variety of scoring functions), Wang *et al.* [116] used a set of configurationally-diverse poses generated with Autodock, and applied the various scoring schemes to rank order the poses. Six of the tested scoring functions (PLP, F-Score, LigScore, DrugScore, LUDI and X-Score) performed better than the Autodock scoring function itself in ranking experimentally observed conformations. The authors concluded that the majority of better-performing score functions in this study were of the empirical type, and, in general, force-field-based score functions were less successful in these tests. Overall, docking scores and experimental binding affinities were poorly correlated. As part of this comparison [116] these authors have also provided a very useful appendix comprising the details of the score functions they compared: Autodock, LigScore, PLP, PMF, LUDI, FlexX, GOLD, DOCK, ChemScore, DrugScore and X-Score [116]. Other docking score functions that may be of interest to the reader include GLIDE [117], DockVision [113, 114], ICM [5], SurFlex [118].

Several other less comprehensive studies have compared various docking methods and/or score functions in the context of virtual screening, and these may be of interest to readers interested in exploring this field. Charifson *et al.* [14], in their initial description of consensus scoring, presented results obtained with DOCK and GAMBLER and then re-scored with different scoring tools. Stahl and Rarey tested different re-scoring procedure on FlexX-derived dockings with seven different target proteins [119], and then Schulz-Gasch and Stahl extended this study to include the GLIDE and FRED docking tools [120]. Finally, Rognan and colleagues have reported studies comparing DOCK, FLEXX and GOLD in the context of virtual screening [53, 121] as well as that of crystal structure reproduction [122].

Consensus scoring has emerged as a powerful retrospective analysis technique, yielding reduced false positive rates in docking-based virtual screening experiments [13, 14]. This approach involves obtaining an output list of dockings with some search engine and *primary* score function, and then re-scoring the final list with various *secondary* score functions and finally taking the intersection of a set of re-scored lists. The relationship between the performance of a given score function in these two roles remains to be established.

## 8. ANALYSIS OF BINDING MODES

As part of the validation of any docking method, it is critical that benchmark calculations be performed to ensure that known ligand poses are reproduced. One would be highly suspicious of a method that had good enrichment without reproducing known binding modes. It is imperative to use a set of carefully chosen protein-ligand complexes,

selecting high resolution structures and discarding ligand structures that defy chemical sense [27]. In this context, the developers of GOLD and FlexX provided an instructive precedent by assembling an extensive test set of experimental protein/ligand complexes and have served the scientific community well by making their test set available to the public [123, 124]. The ability to reproduce known binding modes is typically evaluated by re-docking ligands from co-crystals to the receptor structures from the same co-crystals. It may be beneficial to re-run the calculations with different starting conformations each time and determine the 'success rates' to distinguish sampling problems from scoring problems. Computing accurate binding modes can be a computationally intensive process and as such is not necessarily amenable to screening large databases of compounds. All things being equal, one would prefer the docking program with the highest accuracy in binding mode determination. The pay off in identifying novel binding modes can be huge, as it adds value in suggesting possible modifications to the lead structures.

## 9. COMPARISON OF DOCKING-BASED VIRTUAL SCREENING METHODS

Docking programs can be extremely sophisticated tools that require considerable user experience to be fully exploited. At present this seems reasonable, since the phenomenon being modeled is complex and the forces at play are poorly understood. In docking-based virtual screening the challenge is further exacerbated by the fact that relatively limited computational resource is applied to each receptor-ligand complex, since one would like to screen large numbers of molecules. The problem is difficult, and we want answers quickly.

Both academic and commercial groups have developed tools aimed at solving the docking problem, and many of these tools can be applied to screening (e.g. see section 2). The various methods use different search protocols and different score functions to find optimal docking solutions (see above, esp. sections 2 and 7). Furthermore, the search aspect of docking comprises ligand conformation and protein-ligand configuration (typically the protein is held rigid). At this point in time, it has not been established that a particular search strategy or score function represents a clear advantage over the other methods. The methods and the problem are complex enough that we are forced to evaluate and compare docking methods empirically, based on their ability to reproduce experimental results.

Comparison of published results obtained with different docking methods is difficult due to differences in testing procedures. It is also challenging for a single user to compare several methods, since full mastery of any one method may represent a significant enterprise in itself. If all methods were tested against the same set of target proteins with the same set of actives/inactives, this would go some way toward providing an external "consumer" with a foundation for comparison of methods. However, even given this data, comparison would be difficult. Assurance that the programs have been used comparably is required, and this would seem to dictate that the person(s) making this judgment be intimately familiar with all the programs being

compared. Finally, testing may of necessity be an ongoing task, as each test begs a new test, and methodologies evolve.

The docking problem comprises two sub-problems - searching and scoring [15, 18]. The current thinking seems to be that scoring remains more of a challenge than searching. On the other hand, it has not been established that the search problem has been solved. Exhaustiveness of searching in the context of docking has been systematically examined [125-128], but a recent study concludes that both the searching and scoring aspects of docking remain challenging [116]. When a docking experiment fails, it is important to establish whether a searching or scoring limitation is at fault. This may not be a simple analysis to perform, as tracking of RMSD from a reference structure during the docking experiment may not be possible, and it also may not be possible to perform equivalent docking experiments with different scoring functions.

Studies comparing the performance of different docking programs in the context of virtual screening have not been reported, although some relevant data has been presented in reports that are focused on consensus scoring strategies. The initial description of consensus scoring [14] provides an indirect but useful introduction to the comparison problem. Virtual screening was performed with the two docking programs DOCK and GAMBLER and three therapeutically relevant target proteins. A large number of known actives representing wide activity ranges were used in these tests. The complexity of ligand conformational search was eliminated by using multiple pre-generated ligand conformers and performing rigid-body docking. Consensus scoring was the primary subject of this study, so detailed comparison of the initial docking results based on the primary score functions was not presented, and the diversity of the known active molecules used in the study is also unknown. Similar re-scoring results were obtained when the initial poses were generated with DOCK or GAMBLER, but the authors stated that a more exhaustive comparison was required to draw conclusions about the importance of the primary score function in consensus scoring.

Rognan and colleagues [121] examined virtual screening using two target proteins and three docking programs DOCK, FlexX and GOLD. As in the previous study this report is primarily concerned with analysis of re-scored results, and detailed comparison of initial docking/primary scoring results was not presented. GOLD with the default primary score function performed best at reproducing known binding modes and appears to have the best database screening performance of the three primary docking methods. The performance of secondary score functions was highly target dependent for the two targets described.

Comparison of different docking methods as virtual screening tools represents a significant challenge. The utility of different primary score functions in the context of the same docking search protocol remains to be established, and detailed comparisons of different search methods will also be useful. Pre-generation of ligand conformers followed by rigid-body docking may facilitate comparison of methods by eliminating the effects of different conformational search protocols, but rigid ligand *versus* flexible ligand comparison studies for each method may be warranted.

## 10. APPLICATION OF DOCKING TO NUCLEIC ACID-BASED RECEPTORS

In contrast to proteins, nucleic acids have received much less attention as viable drug targets. Drugs known to interact with DNA include: groove binders (netropsin, daunomycin, etc), intercalators (actinomycin) and alkylating agents (cisplatin) [129]. The variability in DNA structures is relatively small. The folds observed in RNA structures such as ribozymes and ribosomes [130, 131], comparable in complexity to those of proteins, make RNA's attractive as drug targets [132-135]. Very little effort has been devoted to the rational design of ligands for RNA targets. This disconnect has been primarily due to the paucity of available three-dimensional target structures. However, in the last few years a number of crystal and NMR structures of interesting RNA drug targets have appeared in the literature. An important difference between protein and RNA targets relates to binding pocket location. In the case of proteins the binding pocket typically lies rather deep in the interior region and the cavity is well separated from solvent. In RNA targets the binding pocket is located along the surface and is therefore relatively exposed to solvent. The highly charged nature of the target RNA's phosphate backbone requires that electrostatic interactions be handled more accurately than is typically needed for proteins. Scaled partial atomic charges of phosphate groups have been successfully employed to account for solvent screening in docking small molecules to RNA targets\*\*.

Based on DOCK screening of the ACD, aminoglycosides were identified as being capable of binding the standard A-RNA duplex but not the B-DNA form [132]. Structural evidence based on NMR solvent isotope shift measurements indicated that lividomycin, a compound suggested by the calculation, bound to the RNA major groove, corroborating the docking results. In addition, lividomycin caused a significant increase observed in the stability of the RNA duplex ( $T_m = 11.2^\circ \text{C}$ ) while causing no change in the stability of the DNA duplex ( $T_m = 0.0^\circ \text{C}$ ), indicating selective binding to RNA.

Based on computational docking experiments Filikov *et al.* [133] identified lead compounds that disrupt HIV-1 TAR-Tat binding, an interaction necessary for viral replication. In this study ACD compounds were docked to the TAR RNA target structure using a four step docking protocol employing both DOCK and ICM. Two of the eight highest ranked compounds assayed for inhibition of Tat-TAR interaction were found to be active with  $IC_{50}$ 's  $\sim 1 \mu\text{M}$ . Very recent studies from the James group [134, 135] on the same target using DOCK and ICM with an improved scoring scheme produced a sub-micromolar lead with a novel chemotype that demonstrated anti-HIV activity in a cellular assay. NMR studies of the lead compound (acetylpromazine) identified in the above study, showed that it binds specifically to the target at the expected bulge site.

## CONCLUSIONS

In order to derive meaningful conclusions from docking calculations it is beneficial to be aware of the various

approximations employed and their impact on the results. In general one needs to be cognizant of the limitations of the models being used and the potential pitfalls associated with them. It is important to point out that the end-user should pay attention to the documented validation studies performed at various levels of development of a given docking program.

Docking small rigid molecules to receptor structures is rather straightforward (e.g. staurosporine to kinases, steroids to the estrogen receptor, etc.). Ligand flexibility, permutations and combinations of stereoisomers and possible protonation states pose additional challenges to the docking problem, enormously increasing the total number of structures that need to be sampled in a virtual screening experiment. Considering the magnitude of the problem at hand (having to dock millions of molecules for any given receptor), one could justifiably be intimidated. However, by applying intelligent filters the number of molecules that actually needs to be docked can be substantially reduced. The sensitivity of docking programs to the initial ligand conformation is still an open question. The number of ligand conformations that need to be explored during the docking process to qualify as 'exhaustive' has not been established. In addition, for a given target it is not clear how many discrete receptor conformations need to be included in a docking calculation. The good news is that search methods are improving. Better scoring schemes, adequate incorporation of solvent effects and methods to reliably accommodate receptor flexibility are areas of active research that hold much promise.

Specific entropic contributions are largely ignored at present. Parametrization of such effects suffer from the absence of reliable experimental data breaking down the binding free energy into enthalpic and entropic terms. Currently, there is no reliable way to account for the energy differences between receptor-bound and unbound (free) ligand conformations. An indirect way of including this effect has been achieved by an additional 'ad hoc' term in the scoring function that correlates with the number of rotatable bonds in the ligand [133, 136].

Despite all the indicated limitations, significant progress in docking methodology has been made in the recent past. Computational docking calculations are routinely being performed at various stages of the drug discovery process. The power of docking calculations has been well-recognized by interdisciplinary teams in the pharmaceutical industry and tangible results in many cases have been demonstrated. As the field of docking-based virtual screening matures, this recognition will undoubtedly increase. It is hoped that appropriate and widely accepted sets of test data will become established, and that the methods will evolve to facilitate the comparisons required to define the new frontiers.

## ABBREVIATIONS

ACD	=	Available Chemicals Directory
CMC	=	Comprehensive Medicinal Chemistry database
CSD	=	Cambridge Structural Database
GBSA	=	Generalized Born Surface Area
GPCR	=	G-Protein Coupled Receptor

\*\* Mohan V, Smith BA, Griffey RH, McMartin C. COMP-58, 219th ACS National meeting: San Francisco, USA, 2000

LMOD	=	Low Mode Docking
MDDR	=	MDL Drug Data Report
MM/PBSA	=	Molecular Mechanics/ Poisson-Boltzmann Surface Area
NCI	=	National Cancer Institute database
NHR	=	Nuclear Hormone Receptor
RMSD	=	Root Mean Squared Deviation

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