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REVIEW

Protein–Ligand Docking: Current Status and Future Challenges

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ABSTRACT **Understanding the ruling principles whereby protein receptors recognize, interact, and associate with molecular substrates and inhibitors is of paramount importance in drug discovery efforts. Protein–ligand docking aims to predict and rank the structure(s) arising from the association between a given ligand and a target protein of known 3D structure. Despite the breathtaking advances in the field over the last decades and the widespread application of docking methods, several downsides still exist. In particular, protein flexibility—a critical aspect for a thorough understanding of the principles that guide ligand binding in proteins—is a major hurdle in current protein–ligand docking efforts that needs to be more efficiently accounted for. In this review the key concepts of protein–ligand docking methods are outlined, with major emphasis being given to the general strengths and weaknesses that presently characterize this methodology. Despite the size of the field, the principal types of search algorithms and scoring functions are reviewed and the most popular docking tools are briefly depicted. Recent advances that aim to address some of the traditional limitations associated with molecular docking are also described. A selection of hand-picked examples is used to illustrate these features. Proteins 2006;65:15–26.** © 2006 Wiley-Liss, Inc.

Key words: protein–ligand interactions; docking algorithms; scoring functions; flexible docking; docking software

INTRODUCTION

Molecular docking tries to predict the structure of the intermolecular complex formed between two or more constituent molecules. Pioneered during the early 1980s,[1] it remains a field of vigorous research, having become a useful tool in drug discovery efforts, and a primary component in many drug discovery programs.[2] In particular, protein–ligand docking occupies a very special place in the general field of docking, because of its applications in medicine.[3]

From the initial efforts involving the docking of both protein and ligand as rigid bodies,[1] protein–ligand docking has evolved to a level where full or at least partial flexibility on the ligand is commonly employed. Over the last years several important steps beyond this point have been given. Handling efficiently the flexibility of the protein receptor is currently considered one of the major challenges in the field of docking. The fact that proteins are in constant motion between different conformational states with similar energies is still often disregarded in docking studies, even though protein flexibility is known to allow increased affinity to be achieved between a given drug and its target.[4] Furthermore, binding-site location and binding orientation can be greatly influenced by protein flexibility. In fact, X-ray structure determination of protein–ligand complexes frequently reveals ligands with a buried surface area in the range of 70–100%, which can only be achieved as a consequence of protein flexibility.[4]

The historic lock-and-key and induced-fit theories have given their place to more modern theories that bestow a greater weight to the receptor flexibility issue.[4,5] The current paradigm describes a protein as an ensemble of differently populated conformational states in equilibrium, rather than as a clearly dominant more stable conformation,[5] although this may happen in some of the more rigid systems. An important notion to keep in mind is that the highest populated conformations of the receptor in the unbound state are not necessarily the most populated in the protein–ligand complex. In fact, it is seldom the case.[4–6] In terms of docking, these aspects imply that instead of targeting a single pose of a given ligand on a single receptor structure, one should ideally look for the most populated alternatives from an ensemble of solutions comprising several different binding conformations.

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There are currently more than 35,000 crystallographic or NMR structures of proteins or nucleic acids available from the Protein Data Bank (PDB),[7] and the rate of 3D macromolecular structure determination continues to increase every year, particularly with the development of new techniques such as high-throughput X-ray crystallography[20] (see Fig. 1). Moreover, additional momentum has been gained with the sequence of the human genome, offering a rich ground for the generation of 3D structures of biological relevant macromolecules through the application of structural genomics.

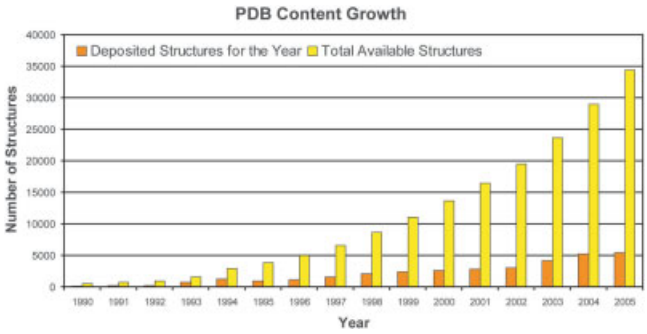

Fig. 1. Protein Data Bank[7] content growth. Number of structures deposited per year and evolution in the number of total available structures.

Many of these macromolecules play vital roles in critical metabolic pathways and may be regarded as potential therapeutic targets, offering unparalleled opportunities for structure-based drug design and discovery. In this context, the accurate prediction of ligand binding modes to macromolecules of known 3D structure is a problem of paramount importance in rational drug design.

Generally speaking, molecular docking comprises the process of generating a model of a complex based on the known 3D structures of its components, free or complexed with other species.[8] In terms of protein–ligand docking methods, the docking problem can be rationalized as the search for the precise ligand conformations and orientations (commonly referred as posing) within a given targeted protein when the structure of the protein is known or can be estimated. The binding affinity prediction problem addresses the question of how well the ligands bind to the protein (scoring).

Docking protocols can be described as a combination of a search algorithm and a scoring function. A relatively large and ever increasing number of search algorithms and scoring functions are available. The search algorithm should allow the degrees of freedom of the protein–ligand

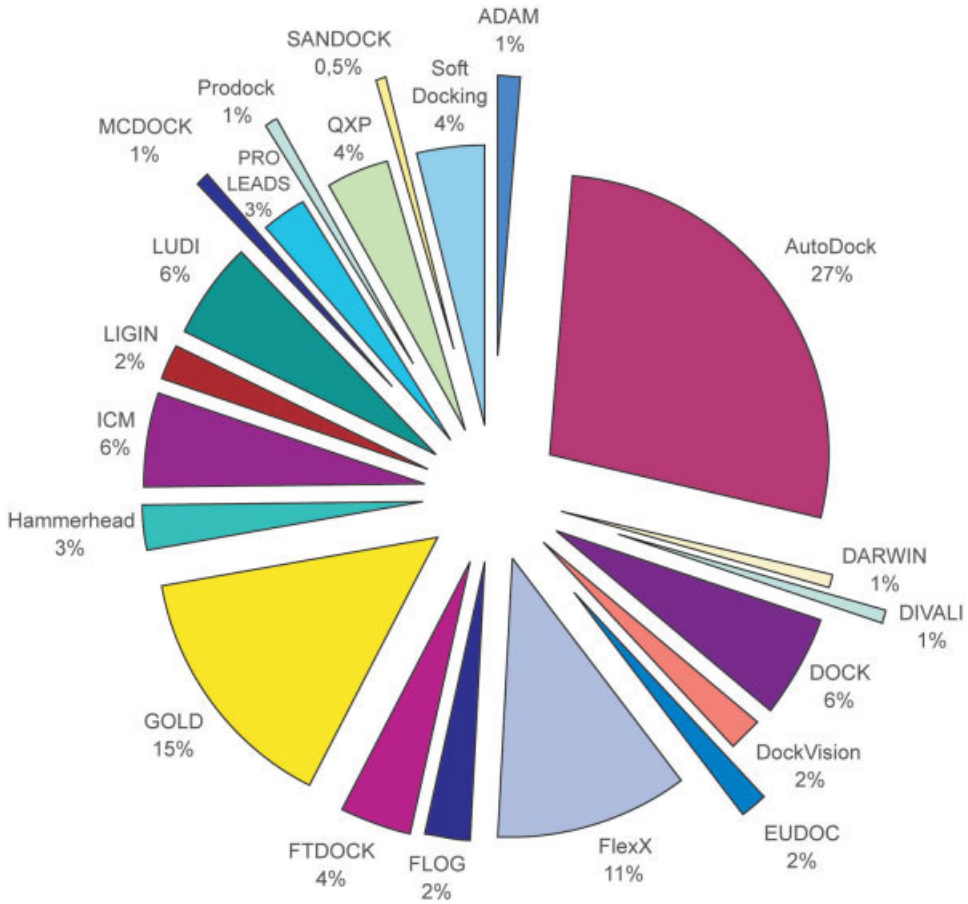

Fig. 2. Docking software—number of citations for some of the most common docking programs, analyzed from ISI Web of Science (2005) considering any of the original references as indicated in Table III.

system to be sampled sufficiently as to include the true binding modes. Naturally, the two critical elements in a search algorithm are speed and effectiveness in covering the relevant conformational space. Among other requirements, the scoring function should represent the thermodynamics of interaction of the protein–ligand system adequately as to distinguish the true binding modes from all the others explored, and to rank them accordingly. Furthermore, it should be fast enough to allow its application to a large number of potential solutions.

Logically, the ideal solution would be to combine the best searching algorithm with the best scoring function. However, several studies have shown that the performance of most docking tools is highly dependent on the specific characteristics of both the binding site and the ligand to be investigated, and that establishing which method would be more suitable in a precise context is almost impossible.[1,9–14]

DOCKING ALGORITHMS

The pose issue in a docking protocol is of maximum importance. The success of a docking algorithm in predicting a ligand binding pose is normally measured in terms of the root-mean-square deviation (RMSD) between the experimentally observed heavy-atom positions of the ligands and the one(s) predicted by the algorithm. The flexibility of the system is a major challenge in the search for the correct pose. The number of degrees of freedom included in the conformational search is a central aspect that determines the searching efficiency.[3]

In a real biological system, the system would include at least the ligand, the macromolecular receptor, and the solvent molecules. Because of the huge number of degrees of freedom associated with the solvent molecules they are normally excluded from the problem, or in special cases implicitly modeled in the scoring functions as a way to address the solvent effect. However, even the remaining part of the system—ligand and receptor—has a computational untreatable number of degrees of freedom, and therefore, the dimensionality of the problem has to be reduced through the application of different approximations, allowing the search space to be more effectively sampled.

There are different levels of approximation. The most basic one is the rigid-body approximation, very popular in early approaches to the docking problem[1] (and still very much applied in the field of protein–protein docking) that treats both the ligand and the receptor as rigid and explores only the 6 degrees of translational and rotational freedom, hence excluding any kind of flexibility. A more common approach these days is modeling ligand flexibility while assuming a rigid protein receptor,[3] therefore considering only the conformational space of the ligand. Ideally, however, protein flexibility should also be taken into account, and some approaches in this regard have been developed.

Flexible Ligand-Search Docking

There are three general categories of algorithms devised to treat ligand flexibility: systematic methods; random or stochastic methods; and simulation methods.

Systematic docking algorithms

The systematic search algorithms try to explore all the degrees of freedom in a molecule, and can be further divided in three main types: conformational search methods, fragmentation methods, and database methods.

Conformational search methods can be seen as the brute force solution to the flexible ligand docking problem. All rotatable bonds in the ligand are systematically rotated through 360° using a fixed increment, until all possible combinations have been generated and evaluated. A major pitfall in this type of methods is that the number of structures generated increases immensely with the number of rotatable bonds, a phenomenon known as the combinatorial explosion. Hence, the application of this type of methods, in its purest form, is very limited. Normally several constraints and restraints on the ligand need to be employed to reduce the dimensionality of the problem.

Fragmentation is one of the most commonly used approaches to introduce ligand flexibility in molecular docking. Fragmentation methods incrementally grow the ligands into the active site, either by docking the several fragments into the active-site and linking them covalently to recreate the initial ligand ("the place-and-join approach"), or by dividing the ligand into a rigid core-fragment that is docked in first place and flexible regions that are subsequently and successively added ("the incremental approach"). LUDI,[15] FlexX,[16] DOCK,[17] ADAM,[18] and Hammerhead[19] are some examples of docking programs that use a fragmentation search method.

Database methods tackle the combinatorial explosion problem by using libraries of pregenerated conformations (conformational ensembles) to deal with the ligand flexibility issue. FLOG,[20] is a typical example of a docking program that makes use of this type of approximation, by generating a small set of 25 database conformations per molecule based on distance geometry, that are subsequently subject to a rigid docking protocol.

Random or stochastic algorithms

Random search algorithms sample the conformational space by performing random changes to a single ligand or a population of ligands. The alteration performed is at each step accepted or rejected based on a predefined probability function. There are three basic types of methods based on random algorithms: Monte Carlo methods (MC), Genetic Algorithm methods (GA), and Tabu Search methods.

In Monte Carlo methods the acceptance criteria for a newly obtained pose is based on a Boltzmann probability function. MC methods have a significant advantage over molecular dynamics methods (MD), as they use a simpler energy function that does not require any sort of derivative information.[21] In addition, MC methods are more efficient in stepping energy barriers, hence allowing more complete searches of the conformation space to be performed. Examples of programs that contain MC-based algorithms include Prodock,[22] ICM,[23] MCDOCK,[24] DockVision,[25] and QXP.[26]

Genetic algorithms apply ideas derived from genetics and the theory of biological evolution to docking. Contrary to the standard MC and Molecular Dynamics (MD) methods, GAs start from an initial population of different conformations of the ligand with respect to the protein. Each conformation is defined by a set of state variables (defined as genes) that describe aspects like the translation, orientation, and conformation of the ligand in relation to the protein receptor. The full set of the ligands state variables is defined as the genotype, whereas the atomic coordinates refer to the phenotype. Genetic operators (mutations, crossovers, and migrations) are applied to the population to sample the conformational space, until a final population that optimizes a predefined fitness function is reached. The programs GOLD,[27,28] AutoDock (version 3.0),[29] DIVALI,[30] and DARWIN[31] all use or include a GA, or a GA-like algorithm.

Tabu search methods operate by imposing restrictions that prevent the search from revisiting already explored areas of the conformational space, promoting the analysis of new regions. This is accomplished through a list that stores previously visited solutions.

The calculation of the RMSD of a new conformation in relation to all previously recorded conformations of the ligand determines if the new conformation is accepted. PRO_LEADS[32,33] is the most popular docking program that uses a Tabu search algorithm.

Simulation methods

Simulation methods employ a rather different approach to the docking problem, and are based on the calculation of the solutions to Newton's equations of motion. Two major types exist: molecular dynamics (MD) and pure energy minimization methods.

Molecular dynamics methods are a powerful and versatile tool in the study of a wide range of applications.[34] However, despite the increasing popularity of MD methods in docking, several pitfalls are well known. In particular, the difficulties in navigating a rugged hypersurface of a biological system and crossing high-energy barriers, and the problem in sampling the conformational space within a feasible simulation period constitute major drawbacks to the application of MD-based methods in protein–ligand docking. Some strategies to compensate these limitations, such as using very high, physically unrealistic temperatures in some parts of the MD simulation, or starting from different ligand positions have been devised.[2] The application of general MD methodologies outside the docking framework, but still in a context of protein-ligand interactions analysis is discussed in detail in the next section.

Energy minimization methods include direct searches (e.g., simplex), gradient methods (e.g., steepest descend), conjugate-gradient methods (e.g., Fletcher-Reeves), second-derivative methods (e.g., Newton-Raphson), and least-squares methods (e.g., Marquardt), and are rarely used as a stand-alone search technique in docking because only local minima can be reached. However, several of the other docking algorithms described above commonly use energy minimization methods, such as the ones mentioned, as a complement. Examples include Prodock,[22] ICM,[23] QXP,[26] DARWIN,[31] DOCK 4.0,[17] ADAM,[18] and Hammerhead.[19]

Flexible Protein Docking

Flexible ligand search docking methods generally give good results for approximately half of the systems they are applied to.[35,36] These success stories comprise systems where the protein target is relatively rigid and the available crystallographic structure of the target is representative of the protein conformation in the docked complex. However, many systems exhibit significant motion upon ligand binding, and even small motions such as the local rearrangement of side chains and the small motion of loops have a deleterious effect on docking results. The development of computational strategies able to accurately account for the flexibility of the protein within the context of the docking problem is still in its infancy, but several approaches able to introduce at least partially flexibility in the protein receptor have been devised. These include some MD and MC methods,[25,29,37] rotamer libraries,[38,39] protein ensemble grids,[40] and soft-receptor modeling.[36,40]

The basic principles outlined above for MD and MC methods within protein–ligand docking with flexible ligands, also hold for flexible receptor docking. Only the dimensions of the problem and of the search space are greatly increased. A growing number of studies have applied these techniques in docking. However, docking simulations with a fully flexible target are currently not feasible, given the need to obtain a docked result with a computational effort of minutes. Some studies with fully flexible proteins have been reported but required several days of computation.[41] Hence, a plethora of different methods have been devised to simplify the molecular description of the system, allowing the incorporation of limited protein motion while keeping computational cost to a minimum. The most relevant ones are outlined below.

The methods based on rotamer libraries try to represent the protein conformational space as a set of experimentally observed and preferred rotameric states for each side chain.[22,42–44] However, focusing on the side chains neglects any real change in the backbone of the protein, and therefore to give a reasonable account of protein flexibility going beyond simple-side chain reorientation is needed.[6]

The use of an ensemble of protein conformations as a target for docking instead of a single structure is viewed as an alternative strategy to deal with protein flexibility. Some different approaches have explored this basic idea,[6,40] but doubts remain on what is the best source of multiple protein structures (crystal structures, NMR, or calculations) and on how to combine the information obtained from the several conformations.[6] Furthermore, some studies of ligand docking to an ensemble of cavities have resulted in worse hit rates than rigid docking itself.[45] FlexE,[46] an extension of the popular FlexX designed to incorporate changes in the receptor structures, is an example of an ensemble docking method.

The Soft-receptor modeling approach combines the information of several different protein conformations (experimental or computationally derived) to generate a single

"energy weighted average" grid, that is subsequently used to dock the ligand(s).[36,40] This approach is relatively cheap in comparison with the other methods already described that include some kind of protein flexibility, as a single energy grid is used as a target for ligand docking. However, it presents some typical disadvantages. In fact, mutual exclusive binding regions can be simultaneously considered, leading to an enlargement of the active-site of the receptor. In addition, this soft-docking approach cannot cope with large scale motion.

The solution for flexible protein–ligand docking for the next years will have to reside in a combination of different methodologies, such as the ensemble docking approach and an induced fit, rather than in the systematic application of the computationally expensive MD methods. Very promising results have already emerged from the application of such combined methods.[47]

SCORING FUNCTIONS

The evaluation and ranking of the ligand conformations predicted on the basis of the search algorithm is a critical aspect of every docking protocol. Being capable of generating the right conformation is not enough. It is also necessary to be able to recognize it. The scoring function should enable the distinction between the true binding modes and all the other alternative modes explored, or between active and random compounds. However, a very rigorous scoring function would be computationally too expensive, rendering the analysis of the several binding modes unfeasible. Hence, a number of assumptions and simplifications have to be used to reduce the complexity of the scoring functions, with a natural cost in terms of accuracy. For this reason, the lack of a suitable scoring function, both in terms of speed and accuracy, is the major bottleneck in docking.[2]

The scoring functions normally employed in protein–ligand docking are generally able to predict binding free energies within 7–10 kJ/mol,[14] and can be divided into three major classes: force field-based, empirical, and knowledge-based scoring functions.

Force Field-Based Scoring

Standard force fields generally quantify the sum of two energies: the interaction energy between the receptor and the ligand, and the internal energy of the ligand. The energies are normally accounted through a combination of a van der Waals with an electrostatic energy terms. A Lennard-Jones potential is used to describe the van der Waals energy term, whereas the electrostatic term is given by a Coulombic formulation with a distance-dependent dielectric function that lessens the contribution from charge–charge interactions.[2,14]

Traditional limitations of force field scoring functions include the absence of solvation and entropic terms, and the inaccurate treatment of the long-range effects involved in binding.

Several force field scoring functions exist. D-Score,[48] G-Score[48] (based on the Tripos force field),[48] GoldScore,[49] and the AutoDock 3.0 scoring function[29] (based on the Amber force field)[50–52] are some of the most traditional examples in terms of docking, but in theory more classical and complete molecular mechanical force-fields such as AMBER (Assisted Model Building and Energy Refinement),[50–52] CHARMM (Chemistry at HARvard Macromolecular Mechanics),[53,54] and GROMOS (Groningen Molecular Simulation System),[55] OPLS (Optimized Potentials for Liquid Simulations)[56] can also be used, with a natural penalty in terms of computational time.

Empirical Scoring Functions

Empirical scoring functions are designed to reproduce experimental data and are based on the idea that binding energies can be approximated by a sum of several individual uncorrelated terms.[2] Experimentally determined binding energies and sometimes a training set of experimentally resolved receptor–ligand complexes are used to determine the coefficients for the various terms by means of a regression analysis.

Interest in empirical scoring functions arises mainly from the typically easy computation of the several terms, while the main disadvantage of these methods rests in their dependence on the experimental data set used in the parameterization process (non versatile and non transferable).

Examples of empirical scoring functions include the Böhm's scoring function (from LUDI),[57,58] F-Score,[16] ChemScore,[59] SCORE,[60,61] Fresno,[62] and X-SCORE.[63]

Knowledge-Based Scoring Functions

Knowledge-based scoring functions focus on following the rules and general principles statistically derived that aim to reproduce experimentally determined structures, instead of binding energies, trying to implicitly capture binding effects that are difficult to model explicitly. Typically, these methods use very simple atomic interactions-pair potentials, allowing large compound databases to be efficiently screened. These potentials are based on the frequency of occurrence of different atom–atom pair contacts and other typical interactions in large datasets of protein–ligand complexes of known structure. Therefore, their derivation is dependent on the information available in limited sets of structures. Muegges's Potential of Mean Force (PMF),[64–66] DrugScore,[67,68] and SMoG score[69] are the most popular examples of knowledge-based scoring functions.

Consensus Scoring

Consensus Scoring[10,13] combines the information obtained from different scores to compensate for errors from individual scoring functions, therefore improving the probability of finding the correct solution. Correlation of the different scoring functions is a problem that should be considered, as it could lead to error amplification instead of error balance. However, despite this possible drawback several studies have demonstrated the success of consensus scoring methods in relation to the use individual functions schemes.[10,14,70] X-CSCORE,[63] which combines the PMF, ChemScore, and FlexX scoring functions with

DOCK-like and GOLD-like algorithms, is an example of a consensus scoring method.

CAPABILITIES AND LIMITATIONS

Docking is currently in a mature stage of development, but it is still far from perfect.[13] Most docking programs now available are normally able to predict known protein-bound poses with averaged accuracies of about 1.5–2 Å with reported success rates in the range of 70–80%.[71–73] However, a significant improvement beyond this range seems for now unachievable,[74] even with the inclusion of receptor flexibility.

The imperfections in the scoring function continue to be the major limiting factor. Scoring functions normally used in docking programs make a number of simplifications and assumptions to allow a more computationally efficient evaluation of ligand affinity, but naturally at the cost of accuracy. In fact, a number of physical phenomena known to be determinant in molecular recognition are completely neglected or at least not fully accounted for in contemporary scoring schemes. Entropy and electrostatic interactions are some examples.[2]

In addition to the scoring problem, a number of other aspects bring additional complexity to a resolution of the general docking problem. The solvent effect and the direct participation of water molecules in protein–ligand interactions, the limited resolutions of most crystallographic targets, and of course, protein flexibility, both in terms of intrinsic structural flexibility and in terms of conformational alterations upon ligand binding, are some of the most relevant ones.[2,21]

Most existing scoring functions neglect solvation effects or simply use solvent models in a snapshot fashion, that is to say, the structures are generated in vacuum and only subsequently ranked with a scoring function that comprises a solvent model. Therefore, in vacuum conformations tend to be favoured. The role played by bound solvent molecules and ions is also normally disregarded, even though the role played by some explicit waters in ligand binding has been well documented.[75–78]

Most of the success stories on docking are based on protein target systems relatively rigid that have a crystallographic structure that is an adequate representation of the protein's conformation in the desired docked complex. However, for many systems a significant rearrangement of the protein takes upon ligand binding. Successfully predicting large-scale protein conformational changes during binding is difficult, and no presently available method can cope with this problem within a reasonable time framework.

Most crystallographic structures have resolution values between 2 and 3 Å, but atomic resolution is only achieved below 1.2 Å.[74] For a resolution of 2.0 Å, for example, the average error in atomic positions can be of 0.3 Å.[79] However, generally speaking, values below 2.2 Å are normally regarded as very good for docking, yielding good results for most typically applications. Nevertheless, one must always bear in mind that at the level of resolution at which most X-ray structures are determined the positions of terminal N and O atoms can normally be assigned only on the basis of a self-consistent hydrogen bond network, and for example, the imidazole ring in histidine can adopt two virtually indistinguishable orientations. Likewise, the positions of hydrogens cannot be directly determined, which has strong implications in assigning the protonation state of some of the ligands and residues, and in predicting the conformation of flexible groups containing H atoms. It must be also kept in mind that the final X-ray structure is only the result of an averaging in time and space of the position of the individual molecules forming the crystal. An additional problem in X-ray structures arises from missing density in specific positions, a problem that does not depend directly on the resolution, and that further complicates structure determination. Therefore, despite the almost invaluable character that X-ray structures of a protein target or a protein–ligand complex can assume in docking, care must be taken when considering them.

Recent trends aiming to improve the level of success of docking involve the inclusion of solvation[80–83] and rotational entropy contributions[10] in the scoring function, and the development of search algorithms more able to describe and efficiently sample the conformational space of the protein–ligand system, within a flexible-target paradigm.[35,45]

SOFTWARE

Establishing a rigorous comparison of protein–ligand docking programs is a daunting task, because it is very difficult to draw conclusions of general applicability.[84] There have been many studies comparing such programs in terms of the accuracy in reproducing the X-ray pose of selected ligands,[19,26,28,29,32,48,49,71,72,83,85–97] the capacity to predict binding free energies from the best-scored pose,[11,12,14,32,49,67,70,74,98–101] and the ability to discriminate known binders from randomly chosen molecules in virtual screening studies.[10,12,14,49,85,87,88,97,100,102,103] However, rationalizing these partial results in terms of the docking programs themselves is very difficult and can be misleading.[84,97] Furthermore, several studies have shown that the performance of most docking tools greatly varies from target to target.[9–12,14] For an excellent, very recent, extensive comparison of some well-known docking tools, see ref. 97.

AutoDock,[29,104,105] GOLD,[27,28] and FlexX[16] are the most popular docking programs, followed by DOCK[17] and ICM,[23] as seen from Figures 2, 3, and 4, but the number of docking programs is high and ever increasing. We would like to stress that these methods are not necessarily the most accurate ones, as accuracy and number of citations do not necessarily correlate. Instead, Figures 2, 3, and 4 give an idea of the most well-established, mature, and widely used methods in the field of docking. This section briefly outlines the basic principles associated with the five most popular ones. From these top five only AutoDock and DOCK are freely available for academic users.

AutoDock

AutoDock 3.0[29] uses a Lamarckian genetic algorithm (LGA), but encompasses also a Monte Carlo simulated

annealing and a traditional genetic algorithm. However, the last two are not as efficient and reliable as the LGA. The program uses a five-term force field-based function loosely based on the AMBER force field, and that comprises a Lennard-Jones 12-6 dispersion term, a directional 12-10 hydrogen bonding term, a coulombic electrostatic potential, an entropic term, and an intermolecular pairwise desolvation term. The scaling factor for each of these five terms is empirically calibrated from a set of 30 structurally known protein–ligand complexes. The free academic licence of AutoDock and the good accuracy and high versatility shown by the program have promoted the widespread use of AutoDock, which explains the very high number of citations. The new version of AutoDock (version 4.0), waiting to be released, also encompasses receptor side chain flexibility in addition to the set of functions already included in AutoDock 3.0.

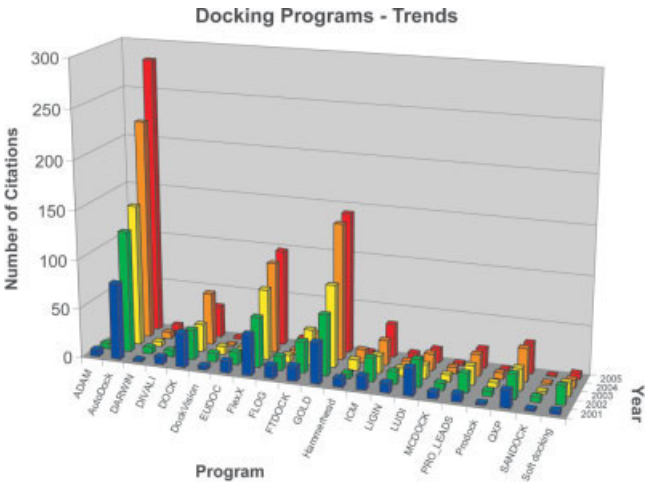

Fig. 3. Docking programs—trends in the number of citations per year for some of the most common docking programs, analyzed from ISI Web of Science (2005) considering any of the original references as indicated in Table III.

GOLD

GOLD[27,28] uses a genetic search algorithm and allows for full ligand flexibility, as well as rotational flexibility for the protein–receptor polar hydrogens. The scoring function is force field based, and includes three terms (a hydrogen bonding term, a 4–8 intermolecular dispersion potential, and a 6–12 intramolecular potential for the internal energy of the ligand). GOLD has one of the most comprehensive test sets from the entire plethora of docking programs, comprising 100 different protein complexes and has achieved a 71% success rate in identifying the experimental binding mode. However, systematic problems in ranking very polar ligands and in ranking general ligands in large cavities have been reported.[71]

FlexX

The fragment-based docking program FlexX[16] is rapidly increasing in terms of popularity. Following the initial selection of the base fragment, conformers are incrementally created using the MIMUMBA torsion angle database,[106] and the conformational space of the ligand is

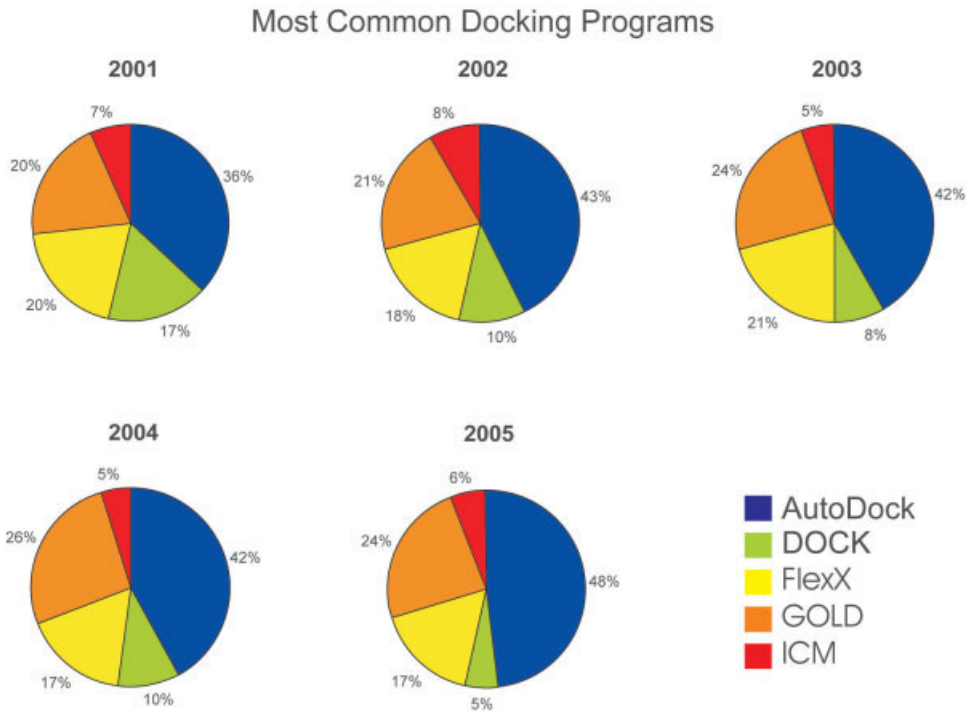

Fig. 4. Most common docking programs—trends in the percentage of citations per year for the five most common docking programs, analyzed from ISI Web of Science considering any of the original references as indicated in Table III.

sampled following a tree-search technique. Final structures are scored using a modified empirical Böhm's scoring function,[58] that includes entropic, hydrogen-bonding, ionic, aromatic, and lipophilic terms, scaled by a corresponding heuristic distance and an angle-dependent penalizing functions.[73] The docking of very flexible ligands is one of the weaknesses of FlexX;[71] however, this program is considerably faster than AutoDock or GOLD.[73]

ICM

The Internal Coordinates Mechanics (ICM) program[23] performs flexible ligand docking using a Monte Carlo minimization procedure in internal coordinates to find the global minimum of the energy function. At each step a torsional or a positional conformational change, followed by a local minimization, is performed. The torsional move can be of the ligand, but also of the receptor side chain, by considering a biased probability method,[23] devised to allow a sampling with a larger probability of the regions of the conformational space known to be highly populated, based on sets of predefined rotamers.[107] The program uses an ECEPP/3 scoring function with a distance-dependent dielectric to perform the local minimization in vacuum. An approximation accounting for the side chain entropies and an electrostatic solvation term—calculated using the MIMEL[108] approximation—are then added, and used to reject or accept a given structure based on the Boltzmann criteria. This program provided the highest accuracy in ligand docking against AutoDock, DOCK, FlexX, and GOLD in a study involving 37 receptors, and has also outperformed DOCK and FlexX in virtual library screening tests.[73]

DOCK

The program DOCK (version 4.0)[17] incrementally constructs the ligand in the binding site step by step, following the choice and placement (following a sphere-matching technique) of an initial rigid anchor fragment. Alternative search algorithms are also available.[96] The program encompasses three different scoring functions, none of which contains explicit hydrogen-bonding terms, solvation/desolvation terms, or hydrophobicity terms, making DOCK a very effective program in fast docking, but with natural limitations in terms of accuracy. Despite this major drawback, DOCK has been shown to handle well small binding sites, opened cavities and small hydrophobic ligands.[71] The treatment of flexible or highly polar ligands have been reported as weaknesses of DOCK.[71] Additional extensions to DOCK allow the treatment of protein flexibility by considering ensembles of protein structures,[40] and the inclusion of a GB/SA continuum model into the scoring function.[109]

EXAMPLES

Ligand Flexibility

Erickson et al.[35] have recently assessed the effect of several key factors on docking accuracy. In particular, they have analyzed the importance of ligand flexibility. A test set of 41 X-ray ligand–protein structures and four docking algorithms—DOCK, FlexX, GOLD, and CDOCKER[110–113]—were considered. For ligands with less than eight rotatable bonds the four methods were shown to perform well, having been able to reproduce the X-ray ligand positions within 2.0 Å heavy atom RMSD for most cases: DOCK (90%); FlexX (80%); GOLD (70%); CDOCKER (95%). However, this study has shown that, except for the CDDOCKER program, the docking accuracy substantially decreases for ligands with eight or more rotatable bonds: DOCK (14%); FlexX (28%); GOLD (19%); CDOCKER (71%). This implies that the ability to distinguish between correct/incorrect conformations for the most common docking programs may decrease for ligands with large numbers of possible low-energy conformations. This is a particular important aspect to bear in mind when docking highly-flexible ligands.

Protein Flexibility

The number of docking studies considering at least partially protein flexibility has been increasing over the past few years. HIV-1 protease (HIVp), an important drug target for the treatment of AIDS,[114,115] extensively studied both experimentally and computationally, and a system where protein flexibility is known to be important for ligand binding, has been frequently regarded as the ideal test case in studies trying to address the protein flexibility issue, because it exhibits a type of flexibility that can pose particularly challenging problems in docking simulations.[36,116,117]

Osterberg et al.[36] have addressed the protein flexibility problem by combining several 3D structures covering a large range of motions of side chains and loops into a single map of interaction energy, thereby incorporating protein motion into the docking simulation with a moderate increase in the computational expense. The method was evaluated considering complexes of 21 peptidomimetic inhibitors with HIVp and was able to accurately address the small but critical motions of side chains in this enzyme. Accounting for the massive rearrangements of the two flaps of HIVp that take place during association with the substrate is out of the scope of the method. The docking of these inhibitors into two energy-weighted maps with AutoDock gave good docked conformations with acceptable predicted free energies for 87% of ligands.

Meagher et al.[116] have developed a receptor-based pharmacophore method that takes into consideration a collection of protein structures MD generated to account for the inherent protein flexibility. This type of models focuses on the consensus regions of a protein, where all the structures exhibit similar requirements, but they do not place any restrictions on the flexible regions of the active site. Considering HIVp as a test case, the authors have observed an increase in the performance of the method as more flexibility on the protein was considered. The method used was able to accurately discriminate between known inhibitors and drug-like noninhibitors (success of 85–90%), and to correctly identify the bound conformations of some ligands, starting from an unbound protein structure.

TABLE I. Main Types of Flexible-Ligand and Flexible-Receptor Search Algorithms

Flexible-Ligand Docking
Systematic
Conformational
Fragmentation
Database
Random / stochastic
Monte Carlo (MC)
Genetic algorithm (GA)
Tabu Search
Simulation methods
Molecular dynamics (MD)
Energy minimization
Flexible-Protein Docking
Molecular dynamics (MD)
Monte Carlo (MC)
Rotamer libraries
Protein-ensemble grids
Soft-receptor modeling

TABLE II. Scoring Functions Most Commonly Used in Docking Programs

Force field based
AutoDock 3.0 Scoring Function[29]
D-Score[48]
GoldScore[49]
G-Score[48]
Empirical
Böhm's Score[57,58]
ChemScore[59]
Fresno[62]
F-Score[16]
SCORE[60,61]
X-Score[63]
Knowledge-based
DrugScore[67,68]
Muegge's PMF Score[64–66]
SMoG[69]
Consensus scoring
X-CSCORE[63]

A protein-specifically adapted function was developed by Radestock et al.[117] This approach uses structural and energetic information about known protein–ligand complexes to tailor knowledge-based pair potentials toward a protein specifically adapted objective function, and implicitly takes into consideration protein flexibility by considering a large number of crystallographic structures of a given enzyme complexed with different inhibitors. In this study, 48 crystallographic determined structures of bound HIVp inhibitors were considered. The method was subsequently validated on a data set of 66 HIVp inhibitors. For ligands with up to 20 rotatable bonds, in more than 75% of all cases a binding mode within an RMSD of 2 Å was identified on the first scoring rank when potentials derived were considered as the objective function in AutoDock, improving the binding mode prediction accuracy with respect to the nonadapted potentials in 14%.

Keizers et al.[118] have automatically docked (using AutoDock and GOLD) and subjected to MD simulations in a cytochrome P450 2D6 (CYP2D6) protein model a series of 3,4-methylenedioxy-*N*-alkylamphetamines. Automatic docking results by themselves were not sufficient to accurately rationalize the experimental binding orientations. However, the MD simulations results with the GROMACS molecular simulation package[119] matched well with the experimental observations. The approach of combining automated docking and MD simulations in a protein model, using the docking results as starting structures for the MD simulation, is a valid strategy to refine the binding orientation of limited sets of docked inhibitors by explicitly considering partial flexibility at the receptor, giving a more accurate account of the distributions of multiple binding conformations in the enzyme.

Explicit Water Molecules

Water molecules can play key roles in protein–ligand docking, either by establishing mediating hydrogen bonds between the protein and the ligand or by being displaced by the ligand. However, despite the crucial importance that water molecules may assume in protein–ligand binding, explicit water molecules are normally not taken into account in docking studies. Accurately and efficiently accounting for the effect of these molecules in protein–ligand docking is one of the major challenges in the field. A relative small number of studies have tried to address this problem.

De Graaf et al.[77] have used AutoDock, FlexX, and GOLD to predict binding modes of ligands in 19 Cytochrome P450 and 19 thymidine kinase protein–ligand crystallographic structures, considering three different scenarios: active sites with X-ray water molecules, active sites with computationally predicted water molecules, and water-free active sites. The results have shown that the consideration of both X-ray and predicted water molecules significantly improved the quality of prediction of the binding modes, leading to better results in terms of RMSD than docking approaches that omit water molecules. A relative average increase of the RMSD accuracy of 18% for AutoDock, 23% for FlexX, and 11% for GOLD was obtained.

Verdonk et al.[78] have implemented a novel approach to score water mediation and displacement in the docking program GOLD. The method in question allows water molecules to switch on and off and to rotate around their three principal axes, adding a constant penalty accounting for the loss of rigid-body entropy for water molecules that are switched on, as a way to promote water displacement. The method was tested on 225 protein–ligand complexes. A small but significant improvement in the quality of the predicted binding modes was observed. Furthermore, the new algorithm was shown to correctly predict water mediation/displacement in 93% of the cases.

SUMMARY AND OUTLOOK

The field of protein–ligand docking has undergone great changes over the past decades. A summary of the current

TABLE III. Some of the Most Common Docking Programs

ADAM[18]
AutoDock[29,104,105]
DARWIN[31]
DIVALI[30]
DOCK 4.0[17]
DockVision[25]
EUDOC[120]
FlexE[46]
FlexX[16]
FLOG[20]
FTDOCK[121]
GOLD[27,28]
Hammerhead[19]
ICM[23]
LIGIN[122]
LUDI[15]
MCDOCK[24]
Prodock[22]
PRO_LEADS[32,33]
QXP[26]
SANDOCK[123]
SFDock[124]
Soft docking[125]

status of the field, major developments underway, present and future challenges, and a brief outline on the most common types of docking algorithms and scoring functions was presented, together with a short description of the most popular docking methods. Only a few years back, docking experiments were based solely in rigid molecules. Today, ligands are commonly treated with partial or full flexibility, and the inclusion of some levels of protein flexibility is becoming a reality. One might envision that in a not very far away future, the treatment of the receptor with full flexibility will be seen as routine procedure.

Despite the very promising picture drawn, molecular docking still holds several hidden weaknesses, and the so-called docking problem is far from being solved. The lack of a suitable scoring function, able to efficiently combine both accuracy and speed, is perhaps the most detrimental weakness. The results of a docking experiment should therefore not be taken as the end result of a structural study, but rather as a good starting point for a deeper and more accurate analysis. In this sense, docking must be necessarily fast, enabling large quantities of data to be considered, and reasonable and coherent solutions to be generated. However, the final result (geometry, ΔG_{bind}) should always be determined by a more accurate and precise methodology, naturally slower.

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