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Protein-Ligand Docking in the New Millennium – A Retrospective of 10 Years in the Field

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Abstract: Protein-ligand docking is currently an important tool in drug discovery efforts and an active area of research that has been the subject of important developments over the last decade. These are well portrayed in the rising number of available protein-ligand docking software programs, increasing level of sophistication of its most recent applications, and growing number of users. While starting by summarizing the key concepts in protein-ligand docking, this article presents an analysis of the evolution of this important field of research over the past decade. Particular attention is given to the massive range of alternatives, in terms of protein-ligand docking software programs currently available. The emerging trends in this field are the subject of special attention, while old established docking alternatives are critically revisited. Current challenges in the field of protein-ligand docking such as the treatment of protein flexibility, the presence of structural water molecules and its effect in docking, and the entropy of binding are dissected and discussed, trying to anticipate the next years in the field.

Keywords: Docking, drug design, entropy, flexibility, scoring, software, virtual Screening.

INTRODUCTION

Protein-ligand docking is a widely used computational tool that tries to predict the most favourable structure of the complex formed between a given protein-target (often an enzyme) and a small-molecule ligand. It can be regarded as part of the more general field of molecular docking, which aims to predict the most favourable structure of the intermolecular complex formed between two or more generic constituent molecules, a definition which also encompasses the field of protein-protein docking [1, 2].

Molecular recognition events are essential in many biological processes, including signal transduction, cell regulation and other macromolecular association actions. These processes rely on a variety of atomic-level scale events including enzyme-substrate, drug-protein, drug-nucleic acid and protein-protein recognition [3], that are of great therapeutic importance. Docking offers a relatively fast and economic alternative to standard experimental techniques, allowing the prediction *in silico* (i.e. computationally) of the binding modes and affinities for molecular recognition events such as the ones outlined above [4]. Within the molecular docking field, protein-ligand docking represents a particularly important and well-established methodology, and a relevant part of the current drug discovery process [1, 2, 5, 6].

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From a functional point of view, docking involves the generation of an ensemble of 3D conformers of a complex starting from the known structures of its free components [7]. In protein-ligand docking this process involves the search through different ligand conformations and orientations (the pose) within a given target protein, and the measure of the binding affinity of the different alternatives (the scoring).

Different poses are generated by a search algorithm, which ideally should sample the degrees of freedom of the protein-ligand complex adequately enough as to include the true binding modes. These different poses are evaluated through a scoring function. This should be able to rank them, and to identify the true binding mode(s) for a given ligand, and to estimate their binding affinity. Hence, a scoring function should be able not only to ensure a distinction between different similar alternatives and ranking them accordingly, but also to represent the thermodynamics of interaction of the protein-ligand system accurately.

Over time different search algorithms have become available, based on quite different approaches. Naturally, the two critical elements in a search algorithm are speed and effectiveness in covering the relevant conformational space [1]. Efficiently dealing with the flexibility is a major challenge, as the computational time associated scales with the number of degrees of freedom included in the conformational search. Several approaches, at different levels of sophistication, have been devised to deal with this issue. These have traditionally been grouped in: rigid-body methods,

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flexible-ligand docking methods, and flexible ligand - flexible target methods.

Rigid-body algorithms comprise the most basic approach to sample the conformational space resulting from a ligand-target association. These methods treat both the ligand and the target as rigid and explore only the six degrees of translation and rotational freedom. For flexible-ligand docking some quite different approaches exist, including systematic, random and stochastic algorithms. Flexible ligand – flexible target methods represent the high-end approach and introduce flexibility in the protein target, in addition to the ligand. As the potential number of degrees of freedom in such a complex is virtually untreatable, several ingenious schemes able to include at least partially, flexibility into the description of the target protein have been developed [8-14]. This topic will be the subject of particular detail in this review.

In terms of scoring functions the number of available alternatives is also quite vast, even though the availability of some scoring functions is sometimes restricted to specific software packages. The most common scoring functions normally applied can be divided into three major classes: force-field-based, empirical, and knowledge-based scoring functions. In addition to good accuracy, an important condition for scoring functions is that they should be fast enough to allow their application to a large number of potential solutions, a feature that implies a number of simplifications that tend to reduce the complexity and computational cost of the scoring functions at the cost of accuracy. Popular examples of scoring functions include ChemScore [15], DrugScore [16, 17], D-Score [18], Fresno [19], F-Score [20], G-Score [18], GoldScore [21], SMoG score [22], and X-SCORE [23].

The best logical solution would seem to be that of comparing the best searching algorithm with the best scoring function. The answer is, however, not so easy, as the performance of most docking tools can be highly dependent on the particular characteristics of the binding site and of the ligands to be investigated. Given the vast number of possible search algorithm/scoring function combinations, establishing which method would be more suitable in a specific context is almost impossible [24-30]. Even though some strategies have been devised to deal with these problems, such as consensus scoring [31], the user's experience continues to be one of the most critical features for the success of a docking study.

The other big factor to take into account is the one thing that connects the user knowledge and experience, the scoring function, the search algorithm, the target, and the ligand(s), and that ideally should be able to get the most out of these components: the protein-ligand docking software program. Over time several studies have tried to evaluate the accuracy of different protein-ligand docking programs. Historically, most of these comparisons have been made in terms of their ability to reproduce the X-ray pose of selected ligands [8, 18, 21, 32-50], their capability to predict binding free energies from the best-scored pose [16, 21, 24, 27, 28, 35, 51-56], or their ability to identify known binders from randomly chosen molecules [21, 24, 27, 29, 47, 48, 50, 56-58]. However, generalizing these partial results in terms of the docking programs themselves is very difficult and often misleading. It is also important to take into consideration that the performance of most docking tools can significantly vary with the particular target under study, and with the particular docking protocol and variables chosen by the user [24, 27-30]. Time is also an important variable to consider, with different software packages working in quite different time-scales. For these reasons establishing a rigorous comparison of protein-ligand docking programs is a daunting task, as it is difficult to draw conclusions of general applicability [59].

CHALLENGES FOR PROTEIN-LIGAND DOCKING

Despite the significant progress that has characterized the past 10 years in the field of protein-ligand docking, several aspects have remained important challenges, with significant margin for improvement. In this section, we review three critical issues for protein-ligand docking: the treatment of protein flexibility, the presence of structural water molecules and its effect in docking, and the entropy of binding.

Treatment of Protein Flexibility

Protein flexibility, including side-chain reorientations and backbone motions, can significantly modulate the geometry and characteristics of the ligand binding site. However, even though most currently available docking methods already treat ligands as flexible, the inclusion of protein flexibility is still a challenging task, remaining one of the most important topics in development within the field of protein ligand docking [60-69]. In fact, although some analogies exist, most of the methods used in the context of ligand flexibility cannot be directly transferred to the protein due to the huge number of degrees of freedom associated [66]. Several strategies to circumvent this problem and to account for protein flexibility, at least at a partial level, have been described in the literature and have gained considerable momentum over the past few years. Most of the strategies already implemented in protein-ligand docking programs account for side chain flexibility only, with the inclusion of backbone flexibility being still in its infancy [62]. Soft docking applications, rotamer exploration approaches, multiple protein structure protocols and molecular dynamics simulation methods represent the main strategies to include some level of protein flexibility into protein-ligand docking.

Soft Docking

Soft docking is a simplistic way to partially introduce receptor flexibility and ligand-induced fit effects. Soft docking methods typically work by allowing a certain overlap between receptor and ligand, normally by tolerant scoring functions, called "soft core potentials". Soft docking methods can efficiently detect subtle conformational changes on the receptor, often not easily perceived through other more sophisticated approaches and do not normally involve an increase in the computational time associated. However, their scope is rather limited to small scale rearrangements associated to side-chain plasticity, without the corresponding backbone adjustment [62, 68].

Rotamer Exploration

Methods based on a systematic exploration of rotamers ensure an effective consideration of side-chain flexibility [62]. Such approaches are typically based on rotamer librar-

ies that try to represent the protein conformational space as a set of experimentally observed and preferred rotameric states for each side chain [10, 70]. Naturally, the application of these methods is in general limited to only a few active site amino acid residues, normally selected by the user. The computational cost associated depends not only on the number of residues subject to rotamer exploration, but also on the size and completeness of the corresponding rotamer libraries. Such approaches present a very useful alternative when tackling receptors for which there is a good structural knowledge on both unbound and bound receptor forms for similar ligands, with such structures suggesting limited structural changes involving only active-site residues. However, focusing on the side chains neglects any real change in the backbone of the receptor, and therefore to give a reasonable account of protein flexibility going beyond simple-side chain reorientation is often required.

Multiple Protein Structures

An alternative way to implicitly introduce flexibility into protein-ligand docking involves the use of an ensemble of protein conformations as a target for docking instead of a single structure. Some different approaches have explored this basic idea [12, 60, 71-76], with alternatives differing on the sources employed to generate multiple protein structures (X-ray crystallographic structures, NMR, molecular dynamics, monte carlo simulations, or elastic network normal mode analysis techniques) and on how information obtained from the several conformations is combined [60, 62, 74-76]. Such approaches typically have a high computational cost, which depends on the number of multiple target structures considered. In addition, they do not enable the generation of novel protein conformations as a result of ligand binding and its exploration of the target conformational space is highly biased and dependent on the set of structures considered as input. Nevertheless such approaches are currently regarded as the most promising routes of future progress [62].

Molecular Dynamics Simulations

The application of molecular dynamics simulations enables an evaluation of side-chain and backbone movement within protein-ligand docking, allowing in principle the generation of novel protein-ligand conformations. However, the practical success of such approaches is still quite small, mainly due to the limited extent of the corresponding MD simulations. In fact, the computational cost required to guarantee a reasonable exploration of the conformational sampling through molecular dynamics simulations is extremely high.

Several studies have applied enhanced sampling techniques to render the application of MD simulations in protein-ligand docking more efficient, involving for example the application of implicit solvent models or the use of geometric constrains on the residues outside the ligand binding region [77-79]. Despite some promising strategies, most applications of molecular dynamics simulations in the field of docking are still done at a post-docking stage, to assess the stability of different docked conformations, to obtain additional conformational and energetic insight into ligand binding, or simply to improve the ligand pose as a refinement tool [80-87].

Presence of Structural Water Molecules

Solvation effects are well-known to influence the binding ability of a drug [69, 88]. As such they have become an integral part of many scoring functions used in protein-ligand docking [2, 3]. Force field-based scoring methods, for example, have long used a distance-dependent dielectric constant to reflect the screening effect of water molecules in electrostatic interactions. In empirical-based scoring methods the inclusion of specific terms related to solvation (e.g. a desolvation energy term) is also quite common, with the corresponding coefficient in the overall energy expression being adjusted to fit binding affinity data from an experimentally determined training set. However, more than solvation, it is the presence of structural water molecules that remains a hard challenge in present day protein-ligand docking.

Water molecules often appear around ligands in protein crystallographic structures, and their presence and precise positioning can lead to significant alterations on both the ligand binding affinity and range of most favored conformations, important issues for protein-ligand docking and virtual screening applications [89-93]. An analysis of a representative set of 392 high-resolution protein-ligand complexes from the Protein Data Bank revealed an average of 4.6 ligand-bound water molecules, 76% of which interacting simultaneously with both the ligand and the protein [94]. For these specific cases, an implicit representation of the solvent is clearly not enough. Hence in general, while part of the function of water in ligand binding can be accounted through a better description of solvation effects, there are a number of important issues that require an explicit atomic level description of water.

In principle, an explicit description of structural water molecules can be done in a number of ways [95]. Typical molecular mechanical force fields contain reasonable water models [96] that can be adopted in protein ligand docking. 3-Point water models, such as TIP3P [97], SPC [98], SPC/E [99], which have a van der Waals center at the water oxygen atom and partial charges at the oxygen and hydrogen atoms are a popular choice. An improved description can be obtained with more sophisticated models, like the TIP4P [100] and TIP5P [101] water models.

In the particular case of protein-ligand docking, through the application of such models, the presence of structural water molecules can be reasonably accounted for in several very precise situations. Imagine, for example, that one is starting a protein-ligand docking (or even a virtual screening campaign) for a protein target on which there is precise information for the presence of a strongly-bound or conserved water molecule (present in a variety of similar X-ray structures for the same target). In such cases, the water molecule can be treated as being an integral part of the protein target for docking. A similar decision can be made regarding docking with ligands containing a common scaffold, when there is an X-ray structure available for one of the ligands in the series showing the presence of a water molecule.

For most situations of interest, however, when no a priori information is available or can be easily obtained, water molecules emerge as an additional participant in docking, often the most elusive one, and an additional variable in the

docking process. While ideally the conformational space associated to the interaction of a variable number of water molecules with a given ligand should be explored together, against a given protein target, and evaluated accordingly, the immense range of possibilities associated greatly limits the practical application of such principles.

An ingenious approach to partially circumvent this issue is the "Just Add Water Molecules" (JAWS) procedure developed by Michel & co-workers [102]. This method uses a double-decoupling scheme to compare the energetic cost associated to water molecule appearance and disappearance on a binding-site grid. Its accuracy in locating hydration sites has been demonstrated for five different biomolecular systems, namely neuraminidase, scytalone dehydratase, major urinary protein 1, β-lactoglobulin, and COX-2. The JAWS methodology has been shown to work particularly well for water molecules well-buried in cavities, in which the grid is isolated from the bulk water. More challenging has been its application to more exposed binding sites, where nevertheless quite reasonable results have been obtained [102]. Other less recent approaches such as AQUARIUS [103], CS-Map [104], MCSS [105], SuperStar [106] and most notably GRID[107] have also been described in the literature to identify potential water binding sites.

Assuming that a good knowledge on the preferred hydration sites is known, either from X-ray or NMR approaches or from computational alternatives such as JAWS, it is necessary for protein-ligand docking to anticipate which water molecules are more likely to be displaced to allow ligand binding. Fast methods like WaterScore [108], HINT [109], or Consolv [110] can be used to differentiate between water molecules that should be included in the docking process and those that should be replaced to make room for the ligand, helping to prepare initial structures for docking. Several docking programs have also implemented strategies to alter water positioning (including its addition or removal) during docking or even after docking, typically through an energy penalty associated [90].

Entropy

It is well known that entropic effects have an important contribution to the protein-ligand binding energy [111-116]. Entropy contributions arise from a variety of aspects. These include the reduction of the translational and rotational degrees of freedom in the ligand, changes in the normal modes of the protein and the ligand during binding, from the arrangement of water layers around the two solutes and even from protonation and deprotonation events [112, 113, 117-122] However, in most commonly used computational applications that deal with protein complexes, including free energy calculations [123, 124], entropy is neglected altogether, or at least the subject of quite dramatic simplifications [114, 125]. In fact, the calculation of the entropic contribution is computationally very expensive as it requires extremely well minimized structures for a Normal Mode analysis, or large numbers of conformations for a Quasi-harmonic analysis [126-128]. This problem is even more striking in the case of protein-ligand docking, for which computational efficiency is an important requirement, with issues like protein flexibility often posing already quite a heavy requirement for a reasonably accurate protocol.

Designing efficient scoring functions able to incorporate entropy is hence a challenge for the next years, although several attempts to include the binding entropy in protein-ligand docking have been reported in the literature, particularly involving re-scoring schemes [117, 129, 130].

Ruvinsky *et al.* [117] have introduced a novel method to estimate the contributions of translational, rotational, and torsional entropy into the protein-ligand binding affinity. The method works by performing multiple docking experiments, clustering the resulting conformations by similarity, and then using a measure of the cluster size to estimate the entropic contribution. Hence, the method assumes that large clusters of conformations are indicative of favorable entropic contributions of the local energy landscapes, and that the docking algorithm provides a reasonable exploration of the associated conformational space. Despite this assumption, this treatment of entropy was shown to improve docking accuracy by 10 –21% when used with the AutoDock scoring function [117]. The authors subsequently showed important improvements when applied in conjunction with other well-known scoring functions [129], namely by 2–25% when used with G-Score, 7–41% with D-Score, 0–8% with LigScore, 1–6% with PLP, 0–12% with LUDI, 2–8% with F-Score, 7–29% with ChemScore, 0–9% with X-Score, 2–19% with PMF, and 1–7% with DrugScore. Tests were performed against a dataset of 100 PDB protein-ligand complexes and ensembles of 101 docked positions generated by Wang *et al.* [131].

Lee *et al.* [130] proposed a similar statistical rescoring method to introduce entropy into the protein-ligand docking problem. According to the method developed by Lee *et al.* a probability function is introduced to analyze the populations of different binding modes in the context of statistical mechanics. This is then used to allow an estimate of the contribution of the state represented by a sampled conformation to the configurational integral, applying the notion of colony energy, proposed by Xiang *et al.* [132]. Improved accuracy in pose prediction has been demonstrated for several common scoring functions, but this method can be easily combined with other preexisting scoring functions, and requires very little extra computational costs because no energy minimizations, dynamics simulations, or clustering is needed [130].

Other attempts to accurately account for entropy involve the inclusion of entropic terms in Knowledge-Based Scoring Functions used in docking [111]. Globally however, the challenge still remains.

PROTEIN-LIGAND DOCKING PROGRAMS

The number of docking programs currently available is high and has been steadily increasing over the last decades. (Table **1**) presents an overview of the most common protein-ligand docking programs, listed alphabetically, with indication of its main citations (original paper), and of the corresponding year of publication and country of origin. This list is comprehensive but not complete. Software programs released in the period 2006-2011 are highlighted and will be

Table 1. Comprehensive List of the Most Common Protein-Ligand Docking Programs

Program	Country[a]	Year[b]	Reference[c]
AADS	India	2011	[133]
ADAM	Japan	1994	[134]
AutoDock	USA	1990	[8, 135, 136]
AutoDock Vina	USA	2010	[137]
BetaDock	South Korea	2011	[138]
DARWIN	USA	2000	[139]
DIVALI	USA	1995	[140]
DOCK	USA	1988	[39, 141-146]
DockVision	Canada	1992	[9]
EADock	Switzerland	2007	[147]
eHiTS	Canada | UK	2006	[148]
EUDOC	USA	2001	[44]
FDS	UK	2003	[38]
FlexE	Germany	2001	[149]
FlexX	Germany	1996	[18, 20]
FLIPDock	USA	2007	[150]
FLOG	USA	1994	[151]
FRED	USA | UK	2003	[49]
FTDOCK	UK	1997	[152]
GEMDOCK	Taiwan	2004	[153]
Glide	USA	2004	[154, 155]
GOLD	UK	1995	[33, 156]
Hammerhead	USA	1996	[157]
ICM-Dock	USA	1997	[41]
Lead finder	Russia | Canada	2008	[158]
LigandFit	USA	2003	[50]
LigDockCSA	South Korea	2011	[159]
LIGIN	Israel | Germany	1996	[34]
LUDI	Germany	1992	[160]
MADAMM	Portugal	2009	[161]
MCDOCK	USA	1999	[162]
MDock	USA	2007	[163]
MolDock	Denmark	2006	[164]
MS-DOCK	France	2008	[165]
ParDOCK	India	2007	[166]
PhDOCK	USA	2003	[167]

(Table 1) contd….

Program	Country[a]	Year[b]	Reference[c]
PLANTS	Belgium | Germany	2006	[168]
PRO_LEADS	UK	1998	[35]
PRODOCK	USA	1999	[169]
ProPose	Germany	2004	[170]
PSI-DOCK	China	2006	[171]
PSO@AUTODOCK	Germany	2007	[172]
PythDock	South Korea	2011	[173]
Q-Dock	USA	2008	[174]
QXP	USA	1997	[175]
SANDOCK	UK	1998	[176]
SFDOCK	China	1999	[177]
SODOCK	Taiwan	2007	[178]
SOFTDocking	USA	1991	[179]
Surflex	USA	2003	[48]
SYSDOC	USA	1994	[71]
VoteDock	Poland	2011	[180]
YUCCA	USA	2005	[181]

[a] Country of origin, as indicated in the author address in the corresponding paper; [b] Programs released in the period 2006-2011 marked in bold; [c] Original main reference considered in the citation analysis.

the subject of particular care, particularly in light of the challenges outlined previously. Special attention will also be dedicated to the docking programs that have been available for longer and that continue to be regarded by users worldwide as a solid and competitive alternative. Finally, a particular look will be dedicated towards protein-ligand docking programs that are emerging as particular promising alternatives and gaining a considerable number of users.

Most Common Docking Alternatives

(Fig. **1**) illustrates the number of citations of the most common protein-ligand docking programs in the period 2001-2011. AutoDock, GOLD, DOCK, FlexX, Glide, FTD OCK and QXP are the most cited docking programs, with over 300 citations each in this period. With the exception of Glide, all the other top cited docking programs have been available since the 1990s. Hence, they may be regarded as well-established mature docking alternatives, with a large and rather stable number of users. LigandFit, Surflex and FlexE are other more recent highly cited docking alternatives.

AutoDock is a versatile protein-ligand docking program developed by Morris & co-workers at the Scripps Research Institute [8, 135, 136]. Its free availability to academic users, together with the good accuracy and high versatility shown, have made it a very popular first choice for new users. These reasons have contributed to its widespread use, well portrayed in the impressively high number of citations in the past 10 years (3980 according to ISI Web of Science). The most recent version - AutoDock 4 (AutoDock Vina is described separately in this review) - includes already side-chain flexibility on selected amino acid residues. AutoDock offers a variety of search algorithms including a Monte Carlo Simulated Annealing algorithm, a Genetic Algorithm (GA), and a hybrid local search GA, also known as the Lamarckian Genetic Algorithm (LGA). The program can be used with a visual interface called AutoDock Tools (ADT) which ensures an efficient analysis of the docking results.

GOLD is another highly regarded protein-ligand docking program. This program is the result of collaboration between the University of Sheffield, GlaxoSmithKline and the Cambridge Crystallographic Data Centre (CCDC), and is commercially available, following the initial development by Jones and co-workers [33, 156]. The program contains a genetic algorithm (GA) based search method for generating ligand poses, a user interface with interactive docking set-up via Hermes, and a comprehensive docking set-up wizard. GOLD allows full ligand flexibility, while ensuring partial protein flexibility, through protein side-chain and backbone flexibility for up to a maximum of ten user-defined residues. The program contains a useful variety of constraint options and allows the automatic consideration of cavity bound water molecules. Several different scoring functions can be considered including GoldScore, ChemScore, Astex Statistical Potential (ASP), and Piecewise Linear Potential (PLP).

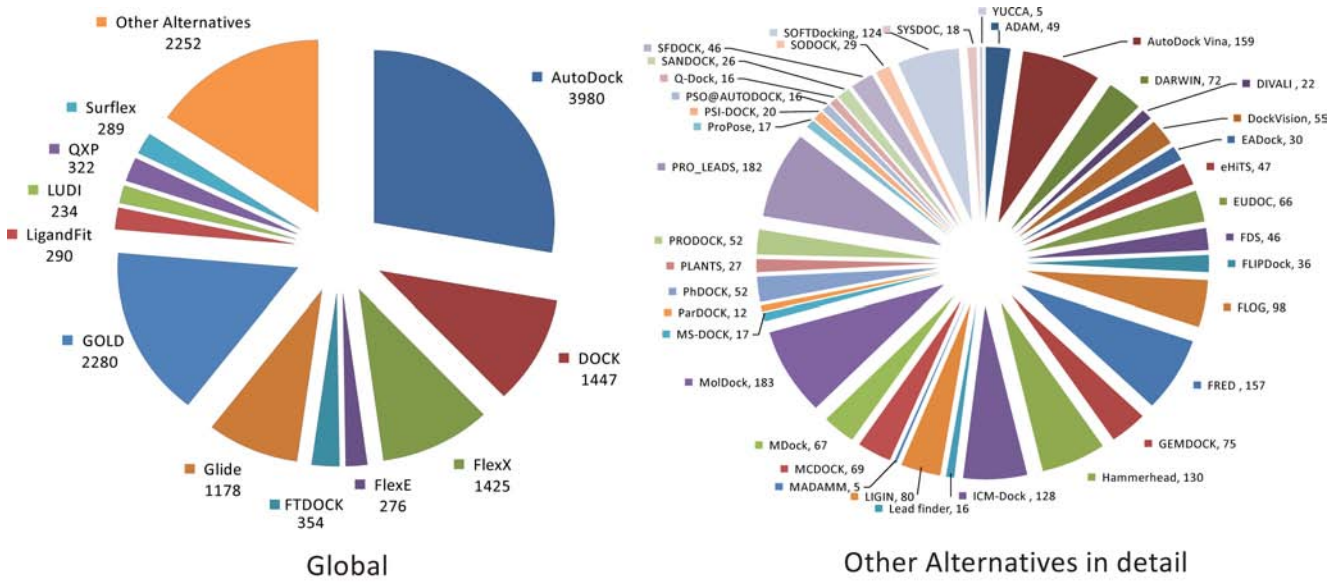

Fig. (1). Number of citations for the most common protein-ligand docking programs in the period 2001-2011. Programs published in 2011 not included.

Extensive options for customizing or implementing new scoring functions through a Scoring Function Application Programming Interface are also present, allowing the user to improve the scoring function to be used in specific receptors.

DOCK [39, 141-146] is a successful docking software initially developed by Irwin Kuntz that has been in the market since 1988 and that is available free of charge for academic institutions. The present version - DOCK 6 - contains a series of improved scoring options including explicit terms for ligand conformational entropy corrections, ligand desolvation, and receptor desolvation. An AMBER molecular mechanics scoring function with implicit solvent, conjugate gradient minimization, and molecular dynamics simulation capabilities are also present.

FlexX [18, 20] (now part of LeadIT) is a very interesting docking program developed by Rarey and co-workers that is presently commercialized by BioSolveIT. FlexX is based on a robust incremental construction algorithm through which the ligand is decomposed into pieces and then flexibly built up in the active site, using diverse placement strategies. The program contains improved capabilities to deal with flexible water molecules and with metal coordination.

Originally developed by Friesner *et al.* [154, 155] in 2004, Glide is a complete solution for protein-ligand docking that is now available as a module in the Schrodinger software suite, commercialized by the Schrodinger LLC. Glide has gained a considerable number of users in just a few years and is emerging as an exciting alternative for protein-ligand docking. Glide generates a set of grids with different types of fields representing geometries and properties of the binding site region of the receptor. The torsional space of the ligand is then exhaustively sampled, generating a large number of binding poses. Following this initial rough positioning, a hierarchical strategy is employed in scoring. This starts with the application of a series of filters that narrows down the range of alternatives to be evaluated, and is followed by a GlideScore scoring, evolving to an in situ minimization with the OPLS-AA force field [182, 183] for the best alternatives. A final energy evaluation with a composite scoring function, which combines empirical and force-field-based terms, is then performed in a selected number of ligand-receptor poses, ensuring a very accurate scoring.

FTDOCK (Fourier Transform rigid-body DOCKing) is a rigid docking program developed by Sternberg & co-workers [152] in 1997 that uses a docking algorithm based on that of Katchalski-Katzir [152]. The program divides the ligand and the receptor into orthogonal grids and scans the translational and rotational space of the two. The scoring method is based in a surface complementary score between the two grids, calculated with the help of Fourier transforms. Although surface complementarity was the only score used in the original method [152], recent versions apply also an electrostatic-based filter [152]. The program is free to both academic and commercial users, but it is no longer supported and no development has taken place in the last decade.

QXP (Quick eXPlore) is a protein-ligand docking application developed by McMarting & Bohacek and originally published in 1997 [32], with a search algorithm derived from the method of Monte Carlo perturbation with energy minimization in the Cartesian space. QXP uses a modified version of the AMBER force field [184, 185], with partial charges calculated from bond-dipole moments [186] and applies a superposition force field that automatically assigns short-range attractive forces to similar atoms within different molecules [187]. After an initial Monte Carlo perturbation, a fast search step is introduced, yielding an approximate low-energy structure prior to energy minimization.

We would like to state very clearly that the number of citations of a given paper is no measure of quality of the corresponding protein-ligand docking software program. It can be taken as much as a rough indicator of the popularity of a specific docking software. Naturally, this popularity reflects mostly

the views of the academic milieu, and only a scarce fraction of the protein-ligand docking applications in the pharmaceutical industry, as most of the research work conducted at the latter is not publicly available and does not get published.

Several features can be associated to this popularity. The price of the program is naturally an important issue. Open source alternatives and programs that are made publicly available to academic institutions tend to get a higher number of citations than the ones that require a paid license. Even within the latter, there can be large differences in price for different software alternatives, which reflect in the number of users, but this can also be affected by the marketing efforts. Another set of issues that are important to the number of citations associated to a given program involves its ease of installation and use, the existence of support and the availability of adequate learning tutorials that could help a user to make the most of the program. Then, on top of all these issues we have, of course, the quality of the program, its range of application, the variety and quality of the available scoring functions and search algorithms, the computational times associated, etc.

Despite these potential limitations, the number of citation, when used with care, presents a useful way to identify and track emerging trends within this rapidly evolving field that is program-ligand docking.

Evolution in the Last 10 Years

(Fig. **2**) shows the evolution of the number of citations per year of the 7 most cited protein-ligand docking programs over the last 10 years, together with its relative percentage in terms of citations per year.

The results show that AutoDock was the top cited protein-ligand docking software throughout the last decade, reaching a level around 500 citations per year. In addition, the results show that while in 2001 its difference towards the second most cited alternative - DOCK - was of only a few citations, in 2010 the difference towards the second most cited docking program – GOLD - grew to close to 200 citations per year. In the past five years, its relative number of citations among the top cited alternatives was maintained among 36-37%, indicating a stable and very significant "market share".

Between 2001 and 2011, DOCK went from being the second most cited program to the fourth place, behind GOLD and Glide, while keeping close to an average number of 150 citations per year. GOLD has been through this period the most cited commercially available docking program. While between 2001 and 2007 GOLD's main competitor among paid alternatives was FlexX, Schrodinger's Glide has emerged as its most cited competitor. Nevertheless, Gold has been able to secure through the past five years a "market-share" of 20-23% among all the most cited alternatives, while Glide is currently at 17% and FlexX at 9%. FTDOCK and QXP only represent 3 and 1% respectively of the total number of citations per year of the seven most cited docking alternatives.

Globally, these results show that AutoDock has been dominating the competition, in terms of number of citations,

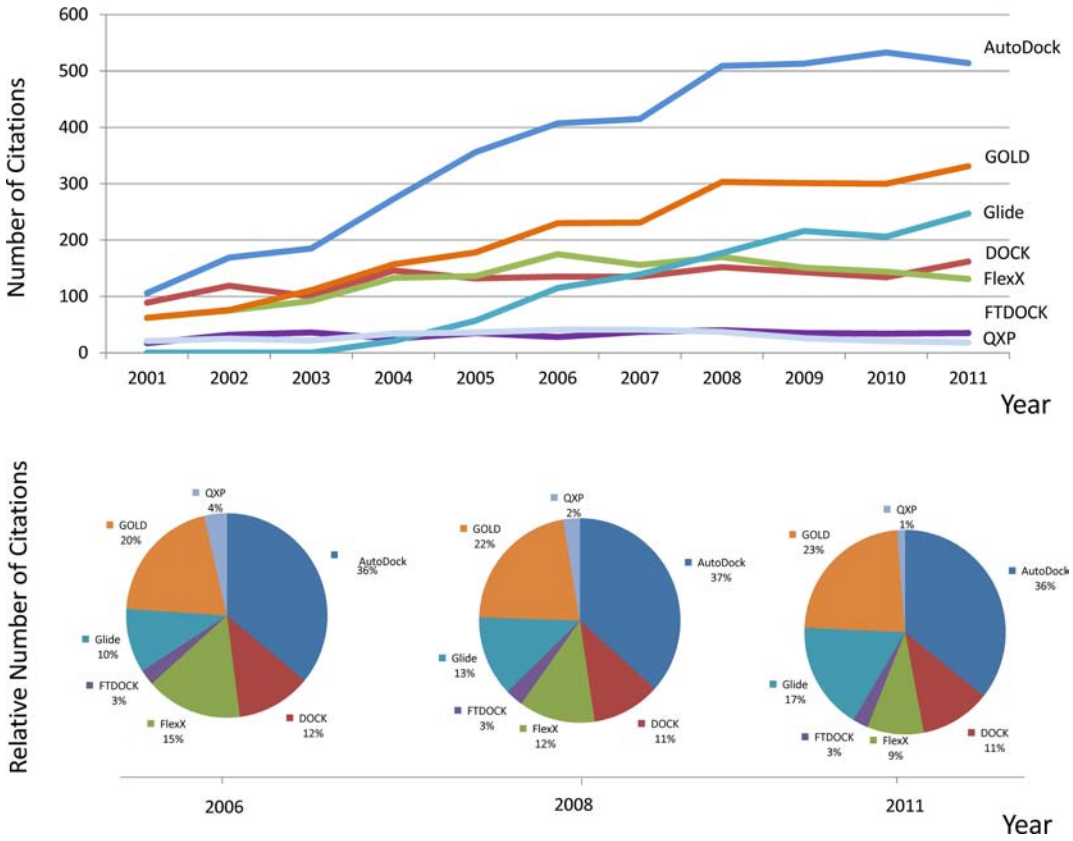

Fig. (2). Evolution of the number of citations per year for the 7 most cited protein-ligand docking programs over the period 2001-2011 and its relative percentage.

and that its number of citations per year and "market share" continues very high. GOLD is a stable second, while DOCK, FlexX, and QXP have been losing "market share". Glide is the fastest growing protein-ligand docking program, in terms of number of citations, among the top 7 alternatives.

Emerging Protein-Ligand Docking Alternatives

In addition to these top cited alternatives other 46 docking programs are mentioned in (Table **1** and Fig. **1**). (Fig. **3**) shows the different proveniences of such alternatives, highlighting the richness of this field of research. In fact, among the docking programs listed in (Table **1**) are creations from 17 different countries from all around the globe. USA, UK and Germany are the countries with the highest number of programs in this field, but in recent years several very appealing alternatives have emerged, particularly in Asia.

AADS (Automated Active site identification, Docking, and Scoring protocol) is an integrated protein-ligand docking tool recently developed by Jayaram and co-workers at the Indian Institute of Technology, New Delhi, India [133]. The program incorporates active site detection, docking, and scoring within a single tool. The AADS methodology is implemented on an 80 processor cluster and presented as a freely accessible web-available tool [133]. The program detects a total of 10 possible binding sites within a target-protein, taking into consideration the physicochemical properties of the amino acid side chains around the possible protein cavities. The program then performs rigid docking of an input ligand/candidate molecule at the 10 predicted binding sites, using an all-atom energy based Monte Carlo method. Scoring is performed through a previously developed in-house scoring function called Bappl (Binding Affinity Prediction of Protein-Ligand) [188] which embeds an effective free energy function, including specific energy terms for electrostatics, van der Waals, hydrophobicity, and loss of conformational entropy of protein side-chains upon ligand binding. Results, including the best four ligand-protein poses and the expected association energy (in kcal/mol) can be emailed back to the user.

BetaDock is a new freely available protein-ligand docking software developed by Kim & co-workers at Hanyang University, Seoul, South Korea [138] and based on the use of Voronoi diagrams. BetaDock differs from other alternatives in the field as it applies a new approach to the protein-ligand docking problem based on the recently developed theory of β-complex and β-shape of molecules, giving higher priority to shape complementarity between a receptor and a ligand [189, 190]. Although the present version is working with rigid ligands only, very promising results have been obtained. In particular, BetaDock was tested against AutoDock 4 (with ligand flexibility turned off) for 85 protein-ligand complexes from the Astex Diverse set database [191], giving superior results, both in terms of the structural quality of the solutions obtained and in terms of speed.

LigDockCSA is a docking program developed by Shin & co-workers [159] at the Seoul National University, in South Korea, that combines a highly efficient search method - Conformational Space Annealing (CSA) - with a scoring function based on the AutoDock energy function with a piecewise linear potential (PLP) torsional energy. Conformational space annealing is designed to search over broad ranges of conformational space, generating numerous local minima before arriving at the global minimum free energy conformation. LigDockCSA applies this principle iteratively, gradually narrowing the conformational space associated to the lower energy conformations. For this reason it is particularly efficient. The performance of LigDockCSA was tested on the Astex diverse set [191] against AutoDock and GOLD, with improved success rates.

ParDOCK (Paralel DOCK) is a web-enabled freely available all-atom energy based Monte Carlo docking program that is implemented as a fully automated, parallel processing mode. The program was developed also by Jayaram and co-workers at the Indian Institute of Technology, New Delhi, India, and takes as initial input a reference complex (including the target protein bound to a reference ligand) and a candidate molecule [166]. The reference complex is automatically taken into consideration in optimizing the conditions for docking the candidate molecule. In this program the geometry of the ligand is optimized with the semi-empirical method AM1 [192], in a process that is followed by a partial charge determination through the AM1-BCC procedure [193, 194]. The General AMBER force field [195], is used to assign atom types, bond angle, dihedral and van der Waals parameters for the ligand. The program was tested on a dataset of 226 protein-ligand complexes through both self-docking and cross-docking, with the authors obtaining the crystal conformation to an average RMSD of 0.53 in 98% of all the cases. Binding site prediction, torsional flexibility of the ligands and protein are some improvements proposed by the authors.

PSI-DOCK (Pose Sensitive Inclined Docking) is a flexible docking method developed by Lai and co-workers [171] at Beijing University, China. The program uses a tabu-enhanced genetic algorithm (TEGA) with a shape complementary scoring function to explore in a first step the potential binding poses of the ligand. The predicted binding poses are then optimized through a competition genetic algorithm and evaluated through a specifically developed improved scoring function (SCORE) to determine the binding pose with the lowest docking energy. For a test dataset of 194 complexes, PSI-DOCK was shown to achieve a 67% success rate (RMSD <2.0 Å) with just a docking run, which was improved to a 74% success rate for 10 runs. The program was also shown to be able to reproduce the binding energy of a training set of 200 protein–ligand complexes with a correlation coefficient of 0.788 and a standard error of 8.13 kJ/mol, while in a test set of 64 complexes a correlation coefficient of 0.777 and standard error of 7.96 kJ/mol were obtained. All protein hydrogen atoms and the flexibility of the terminal protein atoms are intrinsically taken into account in PSI-DOCK. Additionally, there is no need to calculate partial atomic charges, as PSI-DOCK energy function does not contain an electrostatic energy term. These features cancel the need for the user to add hydrogen atoms and restrain the initial docking preparations to a minimum, helping to make this program a particularly easy one to use.

PythDock is a python-based protein-ligand docking program developed by Chung and co-workers [173] at Hanyang University, Ansan, South Korea, that uses a simple scoring

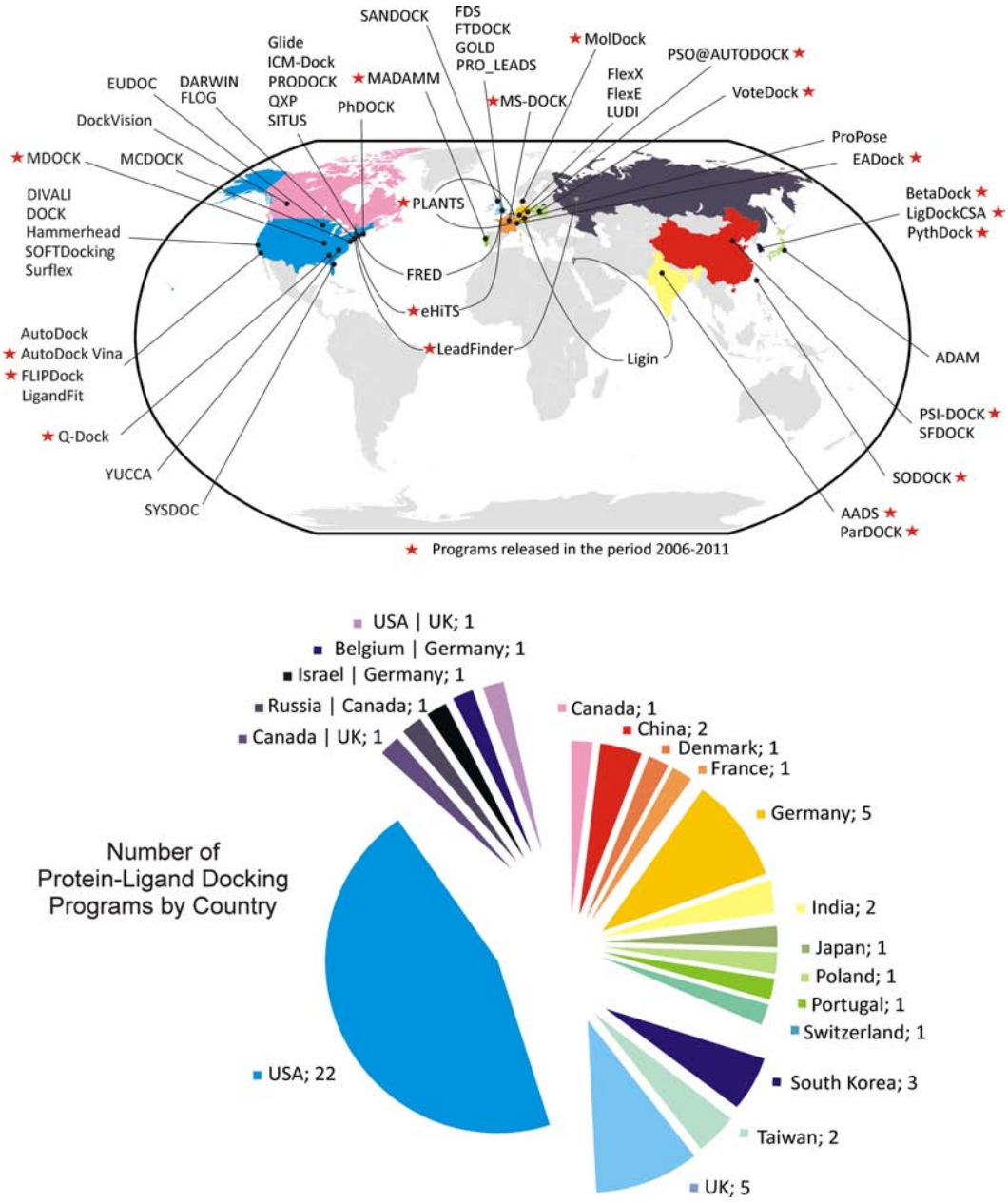

Fig. (3). The World of Protein-Ligand Docking. Distribution of the most common Protein-Ligand Docking programs by country of origin taking into consideration the affiliation of the authors at the time of the publication of the original paper.

function including electrostatic and dispersion/repulsion terms only, together with a search algorithm based on the particle swarm optimization method. The program is a rigid protein-ligand docking program, in the sense that treats ligands and proteins with fixed conformations. A representative number of conformers must be generated using other conformation generating programs prior to docking [173]. Nevertheless, despite its simplicity, the performance of PythDock was evaluated against both AutoDock 4.2 and DOCK 6.2, in a dataset of 14 protein-ligand experimentally determined complexes, giving quite reasonable results [173].

SODOCK (Swarm Optimization for Highly Flexible Protein–Ligand Docking) is a sophisticated protein-ligand docking program developed by Ho and co-workers [178] in Taiwan, specialized in highly flexible ligands. SODOCK contains a novel hybrid search algorithm that couples a Particle Swarm Optimization (PSO)[178] method for solving flexible protein–ligand docking problems with a local search approach. The PSO method used is a population-based search algorithm inspired by the social behaviors of organisms, such as the flocking of birds, simpler and quicker to converge than standard genetic algorithms. The success of PSO is improved with the joint use of the local search algorithm, which is based on the Solis and Wets local search technique [196]. For scoring, SODOCK applies the empirical energy function of AutoDock 3.05. SODOCK has been shown to outperform GOLD 1.2, DOCK 4.0, FlexX 1.8, and AutoDock 3.05 (with a Lamarckian genetic algorithm) in 19 out of a total of 37 ligand-receptor test cases, in terms of RMSD, as reported by

Ho and co-workers [178]. Improvements in the scoring function have been proposed by the authors, as to make this an even more competitive alternative to the treatment of the docking problem.

European protein-ligand docking programs such as Votedock, PSO@AUTODOCK, MolDock, MS-DOCK, MAD AMM, and PLANTS have also been made available in recent years.

VoteDock is a protein-ligand docking program based on a consensus docking approach developed by Plewczynski and coworkers at the University of Warsaw, Poland [180]. The program enables massive ligand-docking to the corresponding targets by applying a combination of several independent docking algorithms and scoring functions, which run in parallel. The method then combines the results from the various programs into a single consensus prediction of the tridimensional structure of the protein-ligand complex. The Seven docking software programs that VoteDock uses, in its consensus approach, are AutoDock 4.2.1, Glide 4.5, GOLD 3.2, Surflex 2.2, FlexX 2.2.1, eHiTS 9.0, and LigandFit 2.3, covering a variety of types of docking algorithms. The performance of this approach was evaluated against an extensive benchmark dataset of 1300 protein–ligands pairs in the PDBbind database for which structural and affinity data was available, with the authors showing that VoteDock is able to dock properly approximately 20% more pairs on average than typical docking methods alone, and 10% more pairs than the single best program tested alone. Despite the fact that most of the individual docking programs required to run VoteDock cannot be distributed under academic license agreement, greatly limiting its availability to standard users, a modified version of VoteDock is in preparation and will be made available through an internet server [180].

PSO@AUTODOCK is a very fast and efficient protein-ligand docking program specifically designed for the treatment of highly flexible ligands and like SODOCK is based on swarm intelligence [172]. PSO@AUTODOCK was developed at the University of Leipzig, Germany, by Namasivayam & Gunther and includes two Particle Swarm Optimization algorithms (varCPSO and varCPSO-Is) designed for the rapid docking of highly flexible ligands. These searching algorithms were embedded in the source code of AutoDock 3 [14]. Hence, PSO@AUTODOCK uses the same energy function that is available in AutoDock 3 (and in SODOCK) for scoring. The main difference resides in the efficiency of the search algorithms developed, with the authors reporting for a selected number of examples, a 10-fold decrease in the number of steps required for identification of the local minimum in comparison with SODOCK, and a 60-fold decrease when comparing with AutoDock 3. These results make PSO@AUTODOCK a very promising alternative for flexible ligand docking, and enable the inclusion of ligand flexibility in virtual screening campaigns of reasonably-sized libraries comprising several thousands of compounds.

MolDock is a docking program developed by Thomsen & Christensen, in Denmark, that is included in the Molegro Virtual Docker package, commercialized by Molegro Aps [164]. MolDock is based on a heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. MolDock automatically identifies potential binding sites, which are then evaluated with the differential evolution search algorithm. The program also applies a scoring function that is an extension of the piecewise linear potential (PLP) introduced by Gehlhaar *et al.* [197]. This new version includes a new hydrogen bonding term that takes directionality into account and an improved electrostatic term with a new charge scheme. The performance of MolDock has been evaluated against 77 protein-ligand complexes from the GOLD dataset [198], resulting, in general, in higher average accuracies than Glide, Surflex, FlexX and GOLD.

MS-DOCK is Multi-Staged docking/scoring protocol [165] developed by Miteva & coworkers at University Paris Descartes, France, based on the program DOCK. The program starts by employing an algorithm called Multiconf-DOCK to generate several conformers per input ligand and then performs a rigid docking of those conformers against the protein target, using DOCK 6.0. In particular, MS-DOCK was specifically designed to allow the rapid screening of a large molecular database, enriching the set of ligands to be effectively evaluated with more sophisticated and expensive methods with molecules having a good shape complementarity for a given target protein binding site. Depending on the target-binding site, MS-DOCK allows the use of only a fraction of the initial database (typically 30-50%) without compromising the performance of a virtual screening protocol in retrieving actives compounds, effectively improving the speed and rate in the search of hit compounds with new scaffolds.

MADAMM (MultistAged Docking with an Automated Molecular Modeling protocol) [161] is a protein-ligand docking application designed by Ramos & co-workers at the University of Porto, Portugal, that allows the flexibilization of both the receptor and the ligand during a multistaged automated hierarchical docking process. MADAMM involves an initial stage in which protein-flexibility is taken into account by using rotamer libraries to generate different combinations of conformers involving the most important amino acid residues at the active-site. From this stage a given target structure can be transformed into as much as 1000 target structures, implicitly accounting for protein flexibility. The program then automatically docks the ligand against each of these target structures using a standard docking program that treats the ligand as flexible, with the current version using GOLD. In the subsequent steps – the automated minimization protocol - a series of energy minimization stages (typically 4) with a molecular mechanics force field (CHARMM) are automatically applied to a selected percentage of the top ranked solutions, with the radius of amino acid residues around the active-site effectively considered in the minimization increasing in each of these steps, as the number of solutions evolving to the next stages is decreasing. Globally, this approach proved to be particularly effective in docking ligands when starting from an unbound structure of the protein. MADAMM is available free of charge.

PLANTS (Protein-Ligand ANT System) [168] is an interesting docking program developed by Korb, Stutzle & Exner at the Universitat Kontanz (Germany) and Universite Libre de Bruxelles (Belgium). This program is based in Ant Colony Optimization (ACO), a methodological approach that

is based on the behavior of real ants on finding the shortest path between their nest and a food source. In the case of protein-ligand docking, an artificial ant colony is employed to find a minimum energy conformation of the ligand in the binding site. These ants are used to mimic the behavior of real ants and mark low energy ligand conformations with pheromone trails. The artificial pheromone trail information is then modified in subsequent iterations to generate low energy conformations with a higher probability [168]. While the ligand is treated as flexible, the flexibility of the protein is only marginally taken into account through the optimization of the atomic position of the hydrogen atoms that are involved in hydrogen bonding. Two specifically designed scoring functions ($PLANTS_{CHEMPLP}$ and $PLANTS_{PLP}$) have also been made available [199]. The program has been shown to reproduce 87% of the complexes present in the Astex diverse set, and 77% of the ones available at CCDC/Astex (non-covalently bound), with root-mean-square deviations of less than 2 angstrom with respect to the experimentally determined structures. PLANTS is available free of charge for academic users.

In addition to these protein-ligand programs, developed in Asia and Europe, several very interesting alternatives have also been developed in the USA. Notable examples include AutoDock Vina, MDOCK, FLIPDock, and Q-Dock.

AutoDock Vina [137] is a new generation docking program developed by Trott & Olson at the Scripps Research Institute, La Jolla, California, following the success of previous AutoDock versions. Like its predecessors AutoDock Vina is freely accessible to a large number of users, as it is open-source. AutoDock Vina inherits some of the ideas and approaches of AutoDock 4, but it is designed in a conceptually different way. It offers significant improvements in the average accuracy of the binding mode predictions, while also being up to two orders of magnitude faster than AutoDock 4. It features also new search and scoring algorithms [137]. Its multi-core capability, high performance and enhanced accuracy, ease of use and free-availability have contributed to an extremely fast dissemination through the docking community, well portrayed in the high number of citations in the first two years after the publication of the original paper. Vina is more than likely to become the most cited docking software in a nearby future. Its high computational efficiency and ability to use multiple CPUs or CPU cores make this program also a very competitive alternative for virtual screening.

MDOCK [163] is a protein-ligand docking software developed by Huang & Zhou at the University of Missouri, USA, that allows the simultaneous docking of ligands against multiple protein structures/conformations, thereby accounting for protein flexibility. The program employs a fast ensemble docking algorithm to account for protein structural variations, which can be applied to different structures for a given target protein taken from the Protein Data Bank (PDB), or to different protein conformations generated from computational methods like molecular dynamics or Monte Carlo simulations, when starting from a single PDB structure. Each protein conformation is treated as an independent target for docking, with the algorithm then automatically selecting the optimal protein conformation. The program uses an iterative knowledge-based scoring function [200, 201] called ITScore that includes only intermolecular interactions. MDOCK was validated on 10 protein ensembles containing 104 crystal structures and 87 ligands, both in terms of binding mode and energy score predictions. An overall success rate of 93% was obtained, when considering as criterion a root-mean-square deviation below 2.5 Å when comparing with the experimentally determined structure. MDOCK package is available free of charge for academic users.

FLIPDock (Flexible LIgand-Protein Docking) [150] is a docking software developed by Zhao & Sanner at the Scripps Research Institute, La Jolla, California that allows the automated docking of flexible ligand molecules into the active site of flexible protein targets. A data structure called Flexibility Tree (FT) [202] is used to represent the conformational space of the receptor and ligand molecules, allowing a hierarchical and multi-resolution representation of conformational changes in macromolecules. In particular, FT breaks down the molecular systems into a set of molecular fragments moving relative to each other, using inter-domain motion descriptors such as hinge, shear, twist, and screw and intra-domain motion descriptors like rotameric side chains, normal modes, and essential dynamics. These descriptions are used to generate a complex subspace involving the most relevant portion of the conformational space of the biomolecular system. A genetic algorithm is employed to search through the solution space in a process that can also involve a two-step divide and conquer algorithm. The current FLIPDock version uses an empirical scoring function based on AutoDock 3.05, but its modular nature and overall architecture of the program offer the ability to incorporate different search algorithms and scoring functions in the future [150]. FLIPDock is particularly strong in handling conformational changes that involve the receptor backbone, when most protein-ligand docking programs fail. The program is free for academic users and will surely become a major docking alternative in the following years.

Q-Dock [174] is a low-resolution flexible ligand docking program with pocket-specific threading restraints developed by Brylinski & Skolnick at Georgia Institute of Technology, Atlanta, USA, designed to deal with the structural inaccuracies in predicted receptor models. Q-Dock describes both the ligand and the protein in a reduced representation mode, i.e. through a coarse-grained knowledge-based potential. Such approach enables the use of low-quality receptor structures, such as the ones routinely produced by proteome-scale protein structure modeling projects, ensuring a wider-range of applicability than typical all-atom approaches. The program uses pocket-specific statistical potentials and harmonic restraints imposed on the binding poses of the common molecule substructures extracted from evolutionarily related proteins. Ligand flexibility is accounted for through an ensemble docking of pre-calculated discrete ligand conformations with Replica Exchange Monte Carlo (REMC). Globally, the authors show that Q-Dock is able to recover on average 25-35% more binding residues and 15-20% more specific native contacts than a variety of commonly used standard all-atom protein-ligand docking approaches in self-docking experiments for a database of 206 X-ray structures.

Performance of Protein-Ligand Docking Programs

As highlighted in the introductory section, comparing docking programs can be difficult. Many studies comparing different docking programs have been made available in the literature. However, the performance of different alternatives can vary significantly with the target, the docking protocol, the specific set of variables, or the user. For these reasons comparisons are not always fair and should be regarded with care. The evaluation of Protein-ligand docking programs against reference validation sets is, in principle, a more trustworthy strategy to assess the quality of different alternatives. Other interesting alternative is the evaluation of the performance of specific docking tools in well-defined structure-prediction challenges, such as the GPCR Dock assessment [203-205]. Here, we review the performance of some of the most common docking alternatives in two specific settings: (1) against the ASTEX diverse set of protein-ligand compounds; (2) against the directory of useful decoys database;

The ASTEX Diverse Set

The ASTEX Diverse Set [191] is a docking validation set, derived from the Protein Data Bank, that contains 85 diverse, relevant protein-ligand complexes. It has become a standard test of reference in terms of pose prediction for docking programs in the last years.

Liebeschuetz *et al.* [206] have evaluated the several scoring functions available in GOLD against this test set. They found that GOLD's ChemPLP was the most effective scoring function for pose prediction in cognate protein–ligand complexes among those available in GOLD, achieving a success rate of 87% over the ASTEX 85 sites below a 2.0 Å RMSD and 68 % below 1.0 Å RMSD. ChemScore, ASP and GoldScore gave sucess rates of 82%, 79% and 78% , respectively, for a 2.0 Å RMSD cut-off, values that decreased to a 53-58% range when a 1.0 Å RMSD criterion was considered.

The performance of DOCK 6.0 against the ASTEX diverse set was analyzed by Brozell and co-workers [207]. Considering as a success criterion a RMSD below 2.0 Å, the authors were able to obtain success rates between 61.4% and 72.4%, depending on the initial starting coordinates used, or the lab where docking was conducted.

GLIDE was also evaluated against the ASTEX set. Repasky *et al.* [208] obtained a success rate of 71% (for a RMSD below 2.0 Å) when using the initial structures taken from the ASTEX set. This success rate was increased to 82% when some improvements were added to the protocol, through the application of the "Schrödinger best-practices" procedure [208], which involved among other issues, the manual inspection and correction of all the bond-orders and charges of the ligands.

Neves & co-workers [209] have analyzed also the performance of ICM against the 85 co-crystal structures of ASTEX. That were able to predict with ICM the top 1 scoring poses below a 2.0 Å RMSD in 91% of the sites with an average RMSD of 0.91 Å (median= 0.54 Å). Predictions below 1 Å and below 0.5 Å were found in 78% and 43% of the cases, respectively.

The Directory of Useful Decoys

The Directory of Useful Decoys (DUD) is a collection of useful decoys for benchmarking virtual screening containing 2950 active ligands for 40 different targets, set by Huang, Shoichet, and Irwin [210]. For each of the active compounds, this database contains a set of 36 "decoys" with similar physical properties, but dissimilar topology, making it a challenging dataset to test protein-ligand docking algorithms.

Using this dataset, the performance of a docking program in this virtual screening procedure is expressed through a graphical representation of the true positive rate versus the false positive rate in terms of receiver operating characteristic (ROC) plots. In ROC plots the True Positive Rate (TPR = TP/P) is plotted versus the False Positive Rate (FPR = FP/N), where TP is the number of True Positives, P is the total number of Positives (actives), FP is the number of False Positives, and N is the total number of Negatives (decoys). An useful measure is the area under the curve (AUC). The higher the AUC value in a ROC curve, the better the discrimination between the true positive and the false positive poses. As a successful docking program in virtual screening should rank active compounds early on a large score list, the fraction of actives recovered at 0.1%, 1% and 2% decoys recovered (abbreviated to $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$) are normally used also as early recognition metrics.

Liebeschuetz *et al.* [206] have evaluated the four scoring functions available in GOLD against the DUD dataset. ChemPLP and ChemScore resulted in average AUC values of 0.70, while ASP gave an AUC of 0.66 and GoldScore of 0.61. ChemPLP showed the best overall performance in the test with $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$ at 8, 14, and 17% respectively. The worst performance was shown by ChemScore with $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$ at 3, 8 and 12%, while ASP and GoldScore exhibited intermediate enrichment factor rates.

Brozell and co-workers [207] have analyzed the performance of DOCK 6.0 against the DUD set and have obtained an average AUC of 0.60 (maximum 0.96; minimum 0.29) with native pairing. True positive rates $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$ at 2.3%, 13.0% and 17.3% were obtained with the default DUD structures, values that increased to 2.6, 15.1 and 20.4% respectively when starting from raw pdb coordinates.

GLIDE was also evaluated against the DUD set by Repasky *et al.* [208], yielding an average AUC of 0.74, a value that increased to 0.80 when using the "Schrödinger best-practices" procedure [208]. Virtual screening experiments with best-practices inputs give true positive rates $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$ at 12%, 25%, and 34 % of known actives, whereas with the default set these recovery rates decrease to 7, 21, and 29 %.

Using ICM against the DUD set, Neves *et al.* [209] were able to obtain an average AUC of 0.72, although the variation between the calculated AUC for the individual templates was quite significant, varying from 0.96 for Neuraminidase to 0.27 for the platelet derived growth factor receptor kinase. True positive rates $ROC_{(0.1\%)}$, $ROC_{(1\%)}$ and $ROC_{(2\%)}$ at 7.3%, 21.0% and 26.6% of true positives, respectively, were ob-

tained using the original pocket coordinates and the default scoring method.

Cross *et al.* [211] have also evaluated the performance of DOCK, FlexX, GLIDE, ICM, PhDOCK, and Surflex against the DUD database. In particular, the authors found that GLIDE (average AUC of 0.72) and Surflex (average AUC of 0.66) outperformed the other docking programs when used for virtual screening (with average AUC values in the range 0.55 - 0.63).

CONCLUSIONS AND OUTLOOK

Over the past decade, protein-ligand docking has emerged as a particular important tool in drug design and development programs. This gain in standing is well portrayed in the rising number of available protein-ligand docking software programs, increasing level of sophistication of its most recent applications, and growing number of users. In spite of the large number of alternatives, we are still far from a perfect docking program. In terms of the searching algorithms, efficiently accounting for protein flexibility remains a challenging task. In terms of the scoring functions features like the presence of structural water molecules and the treatment of entropy, among others, still pose considerable problems for protein-ligand docking. However, the high number of programs, their geographically diverse origin, and the different way in how they deal with the diverse challenges posed by protein-ligand docking are all reasons that demonstrate the vividness of the field.

Many protein-ligand docking programs are currently available and new alternatives are continuing to appear every year. Some of these alternatives will fade among the plethora of protein-ligand docking applications, while others will rise to become top choices among the users of the field. Given the technical development pace in the field all alternatives will eventually become obsolete, at least without a major effort by the development teams in keeping their software programs updated and competitive. Early adopters have the major gain here, even though mastering a new software can be difficult. The richness of this field is sure to make it worth their effort.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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