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Protein-ligand docking

Giovanni Bottegoni

Department of Drug Discovery and Development, Istituto Italiano di Tecnologia, via Morego n.30 Genova, 16163, Italy

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1. ABSTRACT

Ligand-docking is an established computational technique universally applied in structure-based drug design. Since the first attempts carried out in the early '80s to predict the three-dimensional conformation of a protein-ligand bound complex, this methodology has evolved constantly and it is presently implemented in many different ways. The present study aims at explaining the standard protein-ligand docking protocol, together with its main advantages and drawbacks. Milestone reports and future directions are reported and discussed as well.

2. INTRODUCTION

Since the beginning of the 20th Century, recognition at the molecular level has been considered a fundamental step in all biologically relevant processes. Emil Fisher was the first to describe enzyme-substrate interactions using the 'lock and key' metaphor (1). A few years later, Paul Erhlich went further, stating that *"corpora non agunt nisi fixata"* (drugs will not work unless bound). Erhlich was the first to openly challenge the idea that *"corpora non agunt nisi soluta"* (drugs will not work unless in solution), which dated back to the Middle Ages

(2). Since then, the cornerstone of modern medicinal chemistry has been the assumption that complementarity between a small organic molecule and its biological counterpart could explain the potency and the specificity of a drug. However, for many years, knowledge of molecular interactions was limited, difficult to exploit, and played only a marginal role in the quest for new candidates. Drug discovery projects focused on lead compounds selected as result of random screenings or because of their striking resemblance to natural binders. Synthetic campaigns were carried out according to a simple protocol: i) a series of compounds was synthesized introducing a variety of substituents and decorations on a lead; ii) the obtained compounds were tested to estimate a measure of activity, iii) analyzing the experimental results, some simple structure-activity relationships could be gathered, and iv) used to guide the next round of synthesis. The cycle was iterated several times until a promising drug candidate was isolated or the entire project was dropped because of a lack of interesting results. This strategy, which relied largely on synthetic preferences and chemical intuition, was very inefficient and almost impossible to optimize further. A rational approach to drug design began in the 1970s, when: i) advances in molecular biology moved the focus of early experimental tests from cell lines and animals to purified proteins, ii) workstations of unprecedented computational power and storage capability came onto the market, and iii) an ever-increasing number of experimentally solved high resolution protein structures became publicly available (3). Since then, Computer-Assisted Drug Design (CADD) has become a key part of almost every drug discovery program. This is mainly because it is fairly accurate, constantly improving, and, at the same time, faster and much cheaper than *in vitro* experimental setups like combinatorial synthesis and high-throughput screening (4). CADD can be divided into two main branches, depending on whether the coordinates of the receptor are available or not (5). In the latter case, the so-called Ligand-Based Drug Design (LBDD) methods build predictive models by analyzing the chemical and pharmacological features of molecules of known activity. Putative drug candidates that, according to the model, fit the proposed profile of activity are retrieved by mining databases of compounds or synthesizing new molecules from scratch (6-7). When the receptor's three-dimensional (3D) structure is available and can be used to predict ligand-receptor interactions, a Structure-Based Drug Design (SBDD) approach becomes feasible. The general idea behind this approach is that all the information necessary for building a tightly interacting ligand is already contained in the 3D structure of the target. SBDD methods can be further classified into three different groups: manual structure matching, *de novo* design, and molecular docking. The first group is only interesting from a historical perspective: those early attempts were mostly based on interactive exercises carried out on graphical workstations. Despite a limited number of successful applications, they were considered too time-consuming and too dependent on the user's instinct to be of truly practical use (8). *De novo* design methods are based on the assumption that novel highly potent molecules can be produced by growing them directly in the receptor binding site. Molecular fragments are first positioned independently and then joined to form bigger molecules according to the principle of local optimization. Implementations of this basic strategy differ in the way the building fragments are defined and linked, in the way the fitness of the created molecules is evaluated, and in the strategy adopted to efficiently browse the chemical space to avoid a combinatorial explosion. *De novo* design methods have been thoroughly discussed and compared in several recent reviews, to which the interested reader is referred for further details (9-10). The third SBDD approach is molecular docking, which attempts to predict the structure of the intermolecular complex of a given ligand at the receptor binding site by generating and evaluating several conformational variants.

The first automatic docking algorithm was reported in 1982 by Kuntz and coworkers (11). In that pioneer implementation, the docking problem was simply addressed in terms of shape-matching between rigid bodies with no energy evaluation involved. Since then, many other docking programs have been published to provide more accurate predictions of the bound complexes. The representation of the molecules improved, taking full advantage of the ever-increasing computational power available. The typical docking protocol evolved from rigid bodies simulations to include full ligand flexibility and, more recently, partial receptor plasticity. Another fundamental advance was the introduction of scoring schemes that went beyond simple shape complementarity to rank the solutions. Some important milestones in the field are summarized in Table 1.

It is important to note that the general definition of molecular docking covers a heterogeneous ensemble of approaches that vary significantly depending on the chemical and biochemical nature of the ligands and receptors. Researchers have used molecular docking methods to predict complexes formed between proteins (12-13), proteins and nucleic acids (14), nucleic acids and small molecules (15). In this chapter, I will only discuss docking protocols used to predict the binding mode of a drug-like compound at the binding site of a protein. This is because of their relevance in SBDD. The chapter is organized around the general outline of a typical docking exercise. A detailed description of several conformational-searching approaches and scoring schemes used in well-known docking programs will be provided, highlighting their strengths, weaknesses, and open issues. Finally, I will discuss the future directions of the technique together with applications reported in the literature.

3. PROTEIN-LIGAND DOCKING FLOWCHART

The basic idea of predicting the bound pose of a small organic ligand at the target binding site has been used in many different ways. However, we can sketch a common outline of the procedure (see Figure 1). In docking, several problems are addressed sequentially, with each step introducing a new layer of complexity (16-17).

3.1. Receptor structure selection

The predicting power of a docking procedure depends strongly on the quality of the receptor model

Table 1. Some relevant milestones in the development of ligand docking

Year	Event
1894	Emil Fischer proposes the 'Lock and Key' paradigm.
1935	Kirkwood publishes "Statistical Mechanics of Fluid Mixtures."
1937	At Iowa State, Atanasoff and Berry develop ABC, the first fully electronic digital computing machine.
1953	Metropolis and colleagues publish 'Equation of State Calculations by Fast Computing Machines'. The Metropolis Monte Carlo method is born.
1958	Koshland's 'Induced Fit Theory' is published.
1959	Perutz and Kendrew solve the crystal structure of Whale Myoglobin.
1971	At Brookheaven National Laboratory, the Protein Data Bank is created.
1973	Xerox develops Alto™: the first workstation with GUI, mouse, and Ethernet connection.
1975	Levinthal and colleagues report a theoretical model of the interactions map of hemoglobin units in sickle cells fibers.
1977	McCammon, Gelin, and Karplus apply for the first time the principles of molecular dynamics to a macromolecule, the bovin pancreatic trypsin inhibitor.
1981	Connolly provides his definition of a solvent-accessible surface.
1982	Irwin D. Kuntz publishes DOCK, the first modern ligand docking software. The matching routine "*runs to completion in a few hours"* on a DEC PDP 11/70.
1985	Goodford's GRID approach is reported.
1986	DesJarlais introduces ligand flexibility in DOCK.
1990	First version of AutoDock.
1991	Jiang and Kim pioneer the idea of 'Soft' docking.
1992	First version of LUDI.
1994	Di Nola and colleagues apply Molecular Dynamics to the docking problem.
	Leach treats receptor flexibility with the help of side chain rotamer libraries.
	First version of ICM.
1995	Gehlhaar and colleagues propose an evolutionary programming implementation in ligand docking.
	AMBER force field is introduced.
1996	Rejto and Freer challenge the Induced Fit model with the Conformational Funnel Theory.
	First version of FlexX.
	First version of GOLD.
	HammerHead is reported.
1997	Knegtel and colleagues describe an ensemble docking exercise.
	First version of QXP.
	First version of Chemscore.
1998	AutoDock v.3.0 implements the Lamarckian genetic algorithm.
1999	Charifson and colleagues describe the Consensus Scoring approach.
2000	First version of DrugScore.
2002	McCammon's group devises the Relaxed Complex Scheme.
2004	First version of GLIDE.
2007	FITTED and FLIPDock, codes purposely developed for ensemble docking, are reported.
	Hartshorn and colleagues compile the *"Astex"* high-quality test set.
2008	Bottegoni and coworkers report the 4D docking approach.

which, in turn, is affected by the accuracy of the atomic coordinates. Information about a protein's 3D shape comes mainly from experimental structures solved by X-ray diffraction or NMR spectroscopy (18). Currently, over 63,000 X-ray structures are publicly available in the Protein Data Bank (PDB) (19). Crystallographic coordinates within the threshold of 2.5 Å of nominal resolution are usually considered very faithful representations of protein conformations. However, this assumption is not entirely safe, since even high resolution structures can have specific regions where the atomic fit into the electron density map is rather poor. For this reason, the choice of the receptor structure should not be based solely on the resolution but complemented with other metrics such as the R_{free}, the diffraction-component precision index, and the B-factors (20-22). NMR spectroscopy returns low resolution structures and can only be applied to comparatively small proteins. However, NMR spectroscopy, working in solution, can provide a more natural model of the receptor's native state. In some specific cases, NMR conformers can be used as an intuitive representation of protein flexibility (23-24). When an experimentally solved structure is not available, the receptor can be obtained by comparative modeling if sufficient sequence similarity exists (25-26). Successful docking experiments on homology models have been carried out on many different proteins including, but not limited to, protein kinases, hormone receptors, and G-protein coupled receptors (27-29).

3.2. Binding pocket representation

There are three different ways to translate atomic coordinates into a representation of the receptor (30). The most intuitive approach is to express the system in a fully atomistic fashion that explicitly accounts for all the atoms of the ligand and the exposed atoms of the receptor. This approach strongly relies on molecular mechanics force fields to describe atomic radii and charges. Despite being very accurate, an all-atom system is very computationally demanding. This is because the number of interactions to be calculated scales as $O(N^2)$, where N is the total number of atoms. At present, all-atom representations are only used during the final rescoring steps to increase the overall accuracy of the procedure. Thanks to the work of Lee, Richards, and Connolly (31-32), a system can also be represented in terms of interacting molecular surfaces. The solvent-excluded surface is obtained by rolling a spherical probe, which represents a water molecule, on the exposed atoms and then merging the regions of the van der Waals spheres that come in contact with the probe. When flexibility is involved, surface-matching approaches are considered quite impractical and they have been almost completely abandoned except in rigid protein-protein docking. For a detailed description of Connolly surface implementation in ligand docking, see the review by Halperin and colleagues (33). The method most commonly used to describe the receptor is through a set of pre-computed potential grids, according to the methodology

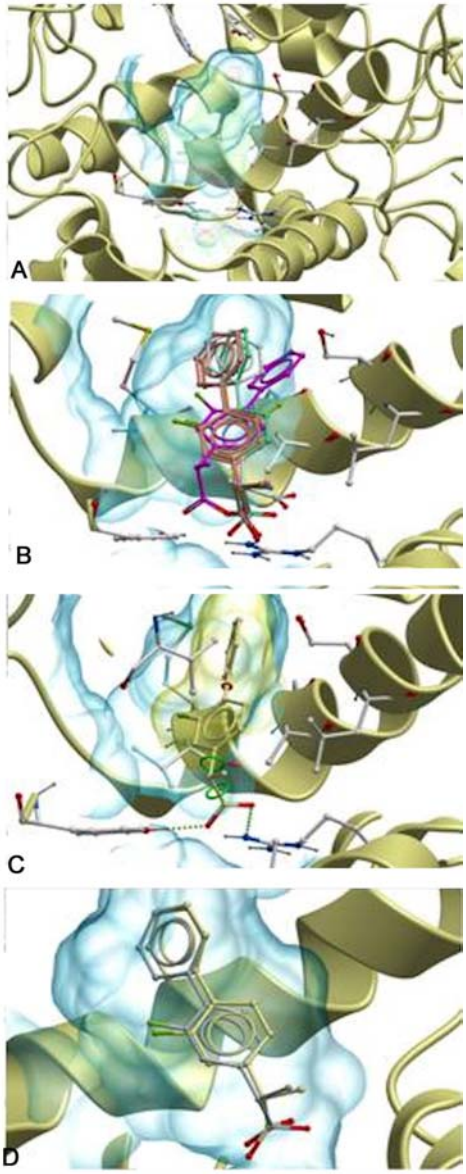

Figure 1. A hypothetical re-docking exercise of flurbiprofen at the binding site COX-2 (PDBid: 3PGH, light green ribbons in the figure) is reported to provide a pictorial representation of four fundamental steps in a protein ligand docking protocol. A) Binding pocket identification (white transparent envelope) and characterization (yellow grid). Some relevant side chains atoms are reported in ball and stick representation. B) Sampling step: different possible binding modes of flurbiprofen are collected. Carbon atoms of each pose are reported in a different color. C) Scoring: for each pose, the contributions of hydrophobic-surfaces-matching (yellow transparent envelope), hydrogen-bonding (dotted lines), and ligand internal strain (green circles) to the binding energy are calculated. D) Ideally, the best scoring pose (green carbon atoms) reproduces faithfully the binding geometry of the native pose (grey carbon atoms) within a threshold of 2 Å (displayed distance 0.5 Å). All pictures were generated with ICM3.6 (Molsoft L.L.C.).

outlined by Goodford in 1985 (34). Storing pre-calculated potential energies arising from interactions between a chemical probe and the receptor, these regularly spaced 3D lattices allow a rapid evaluation of ligand-bound conformations. A basic receptor description can be obtained with just two lattices accounting for van der Waals and electrostatic potentials. However, depending on the specific implementation, contributions from other probes can be mapped as well. Three examples of receptor representations are reported in Figure 2.

3.3. Binding pocket composition

A standard docking procedure does not attempt to consider the whole receptor molecule but rather focuses on a very specific region, the so-called ligand-binding pocket. The binding region has to be defined in terms of both shape and composition. The most straightforward way to define the pocket shape is to select the region immediately surrounding a known ligand co-crystallized in complex with the receptor. Several algorithms have been reported that attempt to predict the pocket location if no holo structure is available, or if the aim of the study is to explore new (e.g. allosteric) spots. The predictive approaches are based on one of the following methods (or a combination of several): analysis of the protein surface and structure, energy profiling, prior knowledge of the substrate, or evolutionary conservation and sequence analysis (35). On average, the predictions are fairly accurate and they all agree that the largest detectable cavity usually corresponds to the binding site. The pocket definition greatly affects the quality of the docking results. If the pocket is too small or shifted from the real location, the accuracy of the docking prediction will be rather poor. If the pocket is too large, the success rate decreases according to its size (21-36). Once the boundaries of the pocket are established, it is important to define the binding site's composition. Usually, in protein crystal structures, the coordinates of hydrogen atoms cannot be solved. They are added to receptor models by purposely developed routines; the polar hydrogen atoms' orientation and the hystidines' tautomerization states should be optimized to reflect the best hydrogen-bonding pattern. Furthermore, other elements should undergo energy optimization, including amidic groups of glutamine and asparagine side chains whose exact orientation is quite hard to gather from diffraction data, side chains from regions that poorly fit into the electron density map, and atoms with high B-factors. Cofactors and metal ions are considered to be part of the receptor cavity and should be included in the definition of the pocket. The role of water molecules is more controversial: some authors suggest that there is no real need for explicit water molecules since their presence can be approximated by cavities in a high distance-dependent dielectric constant (37). Other authors recently reported significant improvements in the quality of results if explicit water molecules are included in the binding site (38-39). A good rule of thumb is to include in the site definition only water molecules that bridge the receptor and a co-crystallized ligand, establishing specific interactions with two non-water molecules.

3.4. Ligand conformational search

During the ligand conformational step, a searching algorithm generates a set of conformational

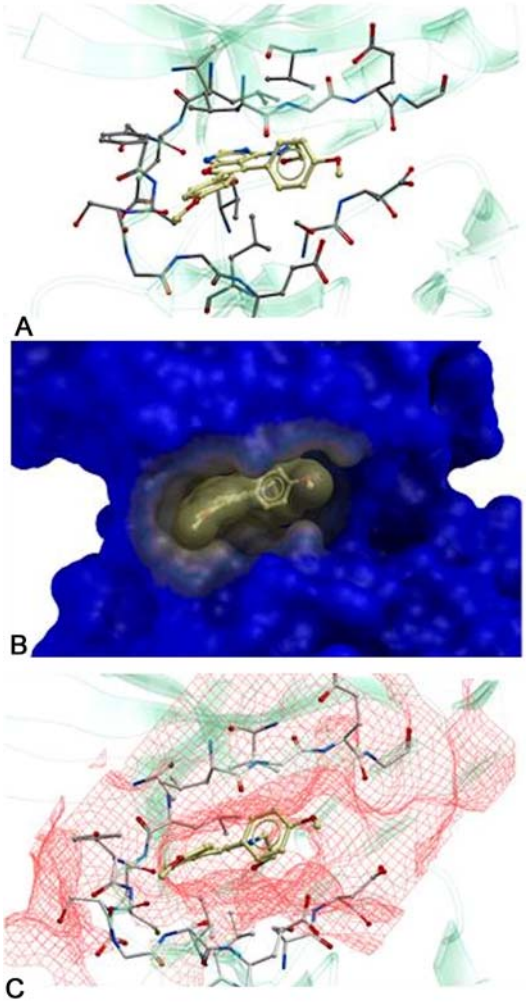

Figure 2. Receptor pocket representations. The bound conformation of thymidine at the thymidine kinase binding site is used as example (PDBid: 1KIM) A) Receptor and ligand atoms are all explicitly accounted for within a given cut-off range (the rest of the protein is reported in transparent ribbons). B) Surface representation: receptor and ligand coordinates are translated in solvent accessible surfaces. The receptor surface is solid blue while the ligand surface is transparent yellow. Ligand atoms are reported in ball and stick representation for clarity. C) Grid-based representation: several features of the receptor atoms can be approximated on regularly spaced points on a grid. The red lattice represents the van der Waals profile of the carbon atoms. Ligand atoms are reported in ball and stick representation.

variants of the ligand at the receptor-binding site. The earliest implementations considered the ligand as a rigid body and only sampled its roto-translational degrees of freedom (8). This approach had a limited application because the conformation that a ligand adopts when bound at the binding site, despite being generally quite close to it, does not always correspond to any energy minima sampled in the solvent (40). However, if ligand flexibility is considered, an accurate sampling of the conformational space quickly becomes too computationally demanding (41). This is because the number of possible conformations scales to the power of the number of rotatable bonds. For this reason, flexible ligand docking protocols adopt different strategies to reduce the exponential dependency of the computational time on the size of the system. Sampling techniques are usually grouped into three main categories: deterministic, stochastic, and simulative methods (16-30-42). Herein follows a detailed discussion of a selection of historically and educationally relevant algorithms. The reader interested in a comprehensive list of reported docking protocols is referred to the meticulous research of Moitessier and colleagues (43).

3.4.1. Deterministic algorithms

In a deterministic approach, the conformational sampling follows a series of steps that will always lead to identical results, if starting from the same state of the system. An exhaustive systematic search is the most basic and intuitive form of deterministic algorithm but, as previously explained, it faces the problem of a combinatorial explosion even when dealing with systems of relatively small size. Deterministic algorithms use heuristics and termination criteria to reduce the size of the conformational space. For example, in the incremental construction algorithm, a ligand is docked at the receptor-binding site in three steps: i) the ligand is divided into a rigid core and flexible fragments, ii) the rigid core is docked at the binding site, and iii) the reconstruction is completed sequentially by adding flexible parts. A well-known incremental construction method is the 'anchor and grow' searching strategy used in DOCK since version 4.0 (44). The ligand is split into fragments concentrically arranged in layers around a rigid anchor; each fragment corresponds to the atoms affected by the torsion of a rotatable bond. First, the anchor is docked using a geometric matching approach. Then, a layer of fragments is added, exploring the associated torsions, optimizing the generated partial poses, and pruning the less energetically favorable conformations. The reconstruction iterates expansion, optimization, and pruning steps for every layer. In order to escape local minima, the pruning strategy is tuned to preserve the diversity of the poses. This strategy is reported to be both accurate and computationally efficient. FlexX uses another incremental construction protocol (45). The rigid core, called the base fragment, is placed at the binding site, evaluating chemical interactions such as hydrogen bonds, salt bridges, and, partly, hydrophobic contributions. The flexible parts of the ligand are added, exploring several preferential values for each torsional angle. Structures that present internal clashes or overlaps with the receptor are eliminated while the remaining poses are subjected to a complete linkage hierarchical-clustering process to eliminate redundancy. The best solutions from each cluster are used to iterate the procedure. In Hammerhead (41). ligand fragments are docked into the binding site and those achieving the highest scores are used as 'heads' to guide the positioning of the rest of the molecule (the 'tail'). Newly generated poses are optimized by energy minimization. Recently, the original strategy used in Hammerhead has been revised, expanded, and included in Surflex (46-47).

3.4.2. Stochastic algorithms

Stochastic algorithms address the ligand conformational sampling as an optimization problem, introducing probabilistic elements like random perturbations on selected parameters (48). Stochastic algorithms for docking can be divided into two groups: genetic algorithms and Monte Carlo implementations.

Genetic algorithms (GA) attempt to find the ligand pose that best fits at the receptor binding site by borrowing strategies (and vocabulary) from evolutionary biology and population dynamics. An initial population of conformations is created randomly, encoding the variables representing each degree of freedom in data structures called chromosomes. Each individual is evaluated according to its fitness for an objective function: a larger fitness corresponds to a greater chance of transmitting its genetic inheritance to the next generation. The better fitting offspring replace the least fit members of the previous generation. To avoid a premature convergence that might trap the system in a local minimum, variations at the chromosome level are introduced randomly in the population by genetic operators such as mutation and crossing-over (49). The Lamarckian GA used in AutoDock is a global evolutionary optimizer equipped with two-point crossing-over and point mutation operators along with a local search feature (50). Before reproduction, each individual undergoes an energy minimization step. Changes introduced locally by minimization are coded back into the chromosome and transmitted to the next generations. This GA was nicknamed 'Lamarckian' after the French biologist Jean Baptiste de Lamarck who introduced the idea (now replaced by Mendelian genetics) that inheritance of acquired traits improves the adaptation of a species to its habitat. GOLD is another docking software whose sampling engine relies on a GA (51). In this case, the evolutionary process does not take place in a single large population. GOLD simulates a distributed environment where multiple subpopulations are handled simultaneously. This scheme, known as the 'Islands model', assumes that each population breeds and evolves separately. However, individual exchanges from one island to another do happen. Migration, a third genetic operator that complements mutation and crossing-over, controls the exchange rate. Population diversity is also preserved by the concept of 'nicheing': two or more individuals share the same niche if the distances between their chromosomes lie within a given threshold. When new individuals join a population, either by breeding or by migration, they replace the least fit individual in their niche rather than in the entire population. The success rate of GAs strongly depends on the quality of the fitting function and the fine-tuning of several parameters (the size of the initial population, crossing-over and mutation rates, number of generations, etc.). If the overall setup creates an adequate evolutionary pressure, successive generations will likely provide at least one individual which represents an optimal bound conformation.

Monte Carlo (MC) implementations apply to the docking problem the general idea of importance sampling, namely the Monte–Carlo-based algorithm conceived by Metropolis and colleagues (52). A random conformation of the ligand is docked at the binding site and, after minimization, the energy score is evaluated. Then, a random change in one or more variables is introduced. The new conformation is minimized and scored again. If the estimated energy is lower than the previous one, the new pose is automatically accepted. If the energy is higher, the Metropolis criterion, a probabilistic test based on a temperature-dependent exponential Boltzmann function, is applied:

Eq.1

$$rand(0,1) \le e^{\left(-\frac{\Delta E}{\kappa_B T}\right)}$$

where ΔE is the energy difference, κ_B is the Boltzmann constant, and T is the temperature of the system. If a randomly generated number between 0 and 1 is lower than the Boltzmann factor, the test is passed and the new conformation is accepted. Otherwise, the new conformation is rejected. This process is iterated until the requested number of cycles is performed. The Internal Coordinates Mechanics (ICM) (53-54) adopts a biased probability stochastic optimizer as a docking engine. The Cartesian coordinates of the system are translated in internal coordinates. Roto-translational variables are sampled, simulating a pseudo-Brownian motion. The sampling of the torsional degrees of freedom is biased toward high probability regions according to a Gaussian distribution. A history term keeps track of the visited regions of the conformational space to help the system escape minima already explored, driving it toward new ones. Before acceptance or rejection according to the Metropolis criterion, a Coniugate-gradient minimization is applied to the generated poses. Glide (Grid-based LIgand Docking with Energetics)(55) is based on a funnel-shaped docking strategy that combines elements from database filtering, systematic search and incremental construction. The rigid core of a ligand is docked at the receptor binding site where the pre-calculated conformations of each rotatable group are evaluated. After a grid-based energy evaluation, MC plays an important role in further optimizing the top scoring conformations to properly orient the more flexible parts. Other docking tools that use MC searches include ProDock (56) and MCDock (57).

3.4.3. Simulation methods

Molecular Dynamics (MD) simulations have been used as docking tools to only a limited extent. In the docking framework, the main limitations of MD are the inability to cross high energy barriers and the amount of calculation time required to perform the simulations (30). Moreover, the final quality of the results is strongly affected by the initial conformation of the system (58). Different solutions have been proposed to more accurately and efficiently explore the energy surface. In MD Docking, receptor, ligand, and solvent are treated at different temperatures, coupling separate regions of the system with different thermal baths (59). Other authors have tried multicanonical MD simulations where the sampling is performed in an artificially flat energy distribution (60).

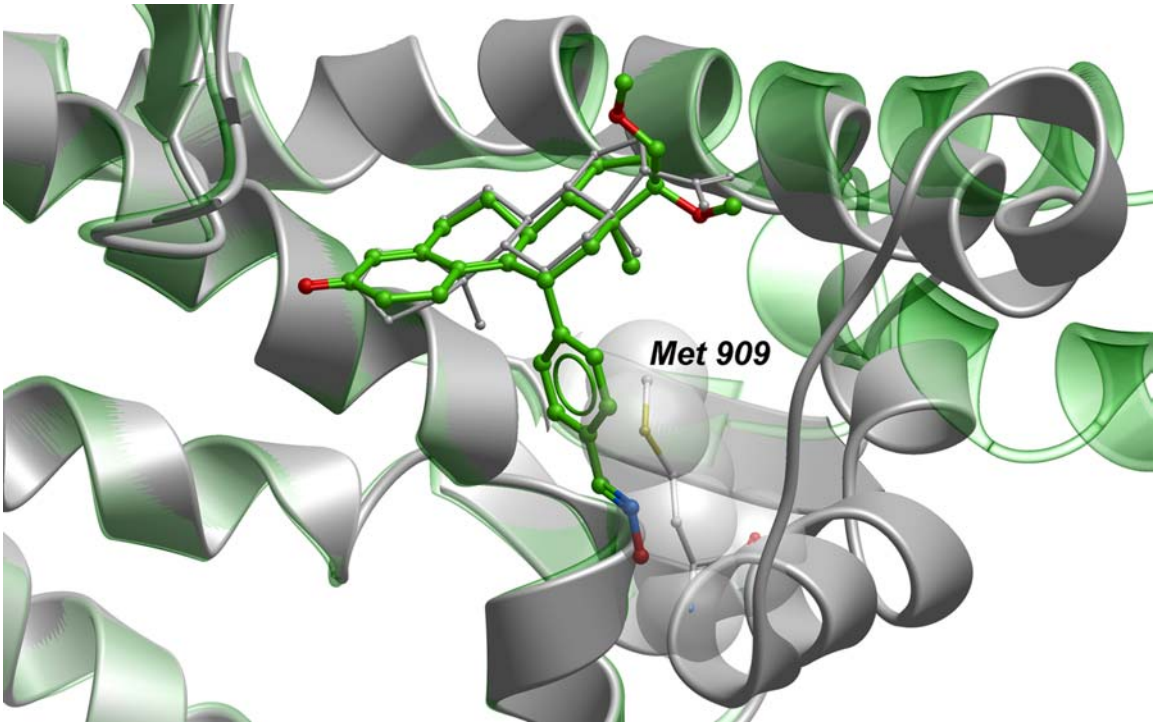

Figure 3. The importance of protein flexibility in cross docking experiments is exemplified by the plasticity of the progesterone-receptor-binding pocket. The modulator asoprisnil cannot be correctly positioned when the conformation from the co-crystal with progesterone is used (white ribbon, PDBid: 1A28), due to a severe steric clash with the side chain of Met909. Asoprisnil is reported in ball and stick representation (green carbon atoms), Met909 side chain atoms are reported explicitly in ball and stick representation and also, to highlight the clash, in transparent CPK. Progesterone (grey ball and stick) and the conformation of the receptor in complex with asoprisnil (green transparent ribbons, PDBid: 2OVH) are reported to facilitate the comparison.

Enhanced sampling methods have also been applied to the docking problem. For example, the protocol proposed by Gervasio *et al.,* the so-called metadynamics protocol, explores the properties of multidimensional free energy surfaces of complex many-body systems using coarse-grained non-Markovian dynamics in the space defined by a few collective coordinates *(61)*. A history-dependent potential term fills the minima in the free energy surfaces, allowing the efficient exploration and accurate determination of the FES as a function of the collective coordinates. Metadynamics is able not only to reproduce a docked pose but also to mimic a ligand exiting or entering a target active site (62).

3.5 .Receptor flexibility

The first model used to explain protein-ligand binding described the event as an interaction between two rigid bodies. The 'lock and key' idea was then replaced by the Induced Fit theory proposed by Koshland: after binding, the ligand modifies the binding pocket to increase its fitness (63). In other words, after binding, the receptor is forced to adopt a conformation which would not exist without the ligand. The Induced Fit paradigm was, in turn, recently superseded by the Conformational Ensemble model (64-65). In this view, proteins naturally exist as an ensemble of interconverting states. The native conformation of a protein is actually an average state resulting from a thermodynamic equilibrium of conformers. When a ligand preferentially binds and stabilizes a receptor variant far from the native state, it triggers a population shift. What is perceived as a local rearrangement of the binding pocket is actually a change in the thermodynamic equilibrium of the whole system. Until recently, almost every docking simulation froze the receptor conformation. Now, a greater understanding of protein-ligand-binding dynamics has led to a gradual introduction of the receptor degrees of freedom in standard docking procedures. The biased strategy used to validate docking protocols probably helped minimize the role of receptor flexibility: when a new tool was proposed, it was usually tested using a re-docking exercise carried out on a set of co-crystals. In re-docking, a ligand is extracted from a holo structure and docked back in the cognate binding pocket. Since the receptor structure is perfectly adapted to accommodate the ligand, the results were quite accurate but, in retrospect, definitely inflated. The importance of protein flexibility became clear when ligands were docked at non-native binding sites (cross docking). In this more realistic representation of a real life scenario, the accuracy of standard docking programs dropped from over 90% to less than 50% (36-66). Figure 3 reports an example of how receptor flexibility can affect a cross docking attempt. Ideally, since even small changes in the binding pocket can considerably affect the final results, receptor and ligand

should be sampled simultaneously in a global energy optimization attempt. This simulation has been described as 'solving the problem of protein folding by adding a ligand'. Because of the very high number of degrees of freedom involved, it would be exceedingly long and would deal with a free energy surface so rugged that convergent results would probably not be obtained (67). The pragmatic strategies adopted to study receptor plasticity can be divided into three main groups: indirect methods, local-variants-based protocols, and multiple receptor conformations docking (MRC).

3.5.1. Indirect methods

Receptor flexibility is accounted for implicitly, allowing a partial overlap between receptor and ligand atoms, and smoothing the high energetic penalties thus generated. The idea of a 'soft' docking algorithm was first proposed by Jiang and Kim (68) and then exploited in several other accounts (69). Soft docking is a computationally efficient and straightforward way of implementing receptor plasticity if the rearrangements to be modelled are local and small.

3.5.2. Local variants generation

The second group includes those methods that, during ligand sampling, introduce a concurrent exploration of some local degrees of freedom of the receptor. These strategies usually deal with torsional angles, which are less likely to dramatically alter the energy profile of the receptor, rather than with planar geometry variables. The torsional search has been reported to be more efficient and prone to converge if carried out in internal coordinates rather than in Cartesian space (70). Local searches can be limited to hydrogen atoms and lone pairs (51) or extended to side chains (71). In the latter case, the side chain flexibility is not modelled continuously but by means of rotamer galleries where the most energetically stable conformers of each amino acid are collected. The discrete nature of these libraries is a good compromise between computational efficiency and accurate results. Several authors have used a two-stage setup where rotamer evaluation coupled with tabu search techniques are followed by a local energy optimization to allow torsional values not originally included in the libraries (72-73). Meiler and Baker adapted the ROSETTADOCK(74) protein–protein docking algorithm to ligand docking (75). Again, side chains are explicitly sampled during a Monte Carlo optimization of the ligand. The novelty here is that the collected rotamer libraries are affected by the conformation of the protein backbone.

3.5.3. Multiple receptor conformations docking

Domain motions, extended loop transitions, and all the rearrangements at the backbone level are far beyond the capabilities of methods that model the binding pocket flexibility on the fly. When direct modelling of local conformational variants is not enough, a multiple receptor conformations (MRC) docking strategy can be attempted. In its most basic form, MRC is just a standard docking approach systematically iterated over an ensemble of receptor conformations. Each conformation is used in an independent simulation and the results are merged together during an additional post-processing step. Members of the ensemble can be collected from experimental structures, generated by computational means, or both (18). Experimental holo structures can be considered reliable representations of those receptor conformational space regions that promote the binding event. Conversely, in-silico-generated conformations can produce unprecedented rearrangements of the binding pocket and, therefore, enhance the possibility of discovering truly novel ligands. Barril and Morley's groundbreaking study of MRC docking (76) suggests that, although using a limited ensemble of selected conformations generally improves the quality of results, an indiscriminate inclusion of a large number of receptor variants in the simulation does not improve the overall performance and may actually be deleterious. In this regard, several strategies have been recommended to select, in advance, a subset of conformations that will most likely provide the best results when combined in an MRC protocol. MRC calculations are time-consuming, since the calculation requirements scale linearly with the number of structures, and they entail a high level of user intervention during post-processing. In order to overcome these two main drawbacks, automatic MRC approaches have been reported, mainly as extensions and adaptations of standard docking engines. In 1997, Knegtel and coworkers (77) proposed an MRC protocol based on DOCK3.5 (78). Separate complements of grids, each one describing a single receptor conformation, were merged into an average model. Huang and Zou (79) describe another MRC approach developed by adapting DOCK (version 4.0) (80). The weighted average method was also applied to several customized versions of AutoDock (81). In particular, the idea of average grids was further improved by introducing Boltzmann weights based on energetic differences (66). In FlexE (82-83), which was developed starting from FlexX, a united protein description is provided: after superimposition, the regions of the receptor conformers that display structural variations are combinatorially merged to generate new states which, in turn, are later used alongside the original structures. MRC studies using Glide (84) and ICM (85-86) were also reported. In particular, the Four-Dimensional docking algorithm considers multiple receptor conformers as an extra dimension of the ligand search space. The procedure combines the accuracy of an MRC implementation with the speed of a single conformer docking. Recently, several algorithms purposely devised for MRC calculations were reported. FITTED (87) is based on a genetic algorithm whose operators can describe receptor flexibility either jumping among different protein conformations from the set (semi-flexible run) or rearranging side chains and backbone variables independently. FLIPDock (88) simultaneously codes ligand and receptor motions in a high level data structure, the Flexibility Tree, originally developed to describe conformational subspaces of macromolecules and later adapted to the docking problem. One of the most interesting features of the Flexibility Tree is that experimental evidence and biological expertise can be included quite easily in the flowchart. In gapped models (36-89), an ensemble of receptor variants is generated by deleting different parts of the receptor, typically converting one or more binding pocket side chains into alanine. Empty

spaces allow an initial positioning of the ligand, avoiding severe steric clashes. The receptor is then returned to the ungapped state and the complex undergoes geometrical optimization before rescoring. Gapped models combine features from MRC and local variant optimization techniques.

The Relaxed Complex Method (RCM) developed by McCammon's group is a good example of how an advanced MRC protocol can contribute to the success of rational drug design efforts (90). In RCM, MRC docking is complemented with other computational techniques in an advanced protocol that provides a reliable prediction of a ligand-binding mode. First, the receptor flexibility is explored in a fully atomistic fashion using long MD simulations. Plain MD or enhanced sampling strategies can be used to sample the receptor conformational space. Several snapshots are extracted from the trajectory and used as receptor conformers. In the first applications of RCS, snapshots were extracted at equal time intervals while later implementations strongly rely on advanced cluster analysis algorithms to eliminate conformational redundancy and to reduce the computational burden (91). The docking step is accomplished using AutoDock Lamarckian GA, which takes full advantage of the improved desolvation term introduced since version 4.0 (92). The most promising poses are rescored with a customized implementation of MM-PBSA, an end-point free energy assessment approach (93-94). In standard MM-PBSA, an MD simulation of the bound complex is performed to calculate, according to molecular mechanics (MM), the contribution of ligand receptor direct interactions. The solvation energy is decomposed in electrostatic and non-polar components: the electrostatic contribution is retrieved by solving the Poisson Boltzmann (PB) equation in a continuum solvent model, while the non-polar effect is estimated according to the surface area (SA) accessible to the solvent (95). In RCS, MM-PBSA is modified to include the unfavorable entropic contribution to the binding event due to the loss of roto-translational and conformational entropy (96). The reference states for the unbound protein and ligand are extracted from the docked complex trajectory: what may appear as an oversimplification significantly reduces computational requests, decreases convergence issues, and introduces only negligible variations in the final outcome. RCS has successfully helped in the search for novel inhibitors of HIV Integrase (97), Kinetoplastid RNA Editing Ligase 1 (KREL1) of *T.brucei* (91), AChBP (98), and MMP-2 (99).

3.6. Scoring

In the last stage of a docking protocol, the poses retrieved during sampling need to be evaluated in terms of interaction energy with the receptor. The quantitative estimate of the binding affinity is usually reported in terms of Gibbs free energy difference (ΔG_{bind}) between receptor (R) and ligand (L) in their unbound state and the complex (RL) formed upon binding. From the statistical thermodynamics point of view, the theoretical frame for evaluating the free energy of binding is well established (100-101). Receptor ligand associations are usually regarded as an event that combines enthalpic and entropic effects. There is an electrostatic component that accounts for basic interactions, such as the H-bond formation and the Coulombic attraction/repulsion among charges, as well as for superior order contributions such as dipole-dipole interactions. Shape complementarity is also accounted for by van der Waals interactions. In physiological conditions, the binding event takes place in solvent and, for this reason, the contributions of hydrophobic surfaces solvation and desolvation must be considered too. When bound, receptor and ligand can only adopt a narrower range of conformations as compared to the unbound states, intuitively decreasing the entropy of the system. Finally, the ligand could adopt strained conformations, directly increasing the system's potential energy. All these contributions are expressed by ΔG_{bind} which, in turn, is related to the equilibrium binding constant K_{eq} according to the following equation:

Eq.2

$$\Delta G_{bind} = G_{RL} - G_R - G_L = \Delta H_{bind} - T\Delta S_{bind} = -RTln(K_{eq})$$

where T is the temperature of the system.

ΔG_{bind} can be determined from first principles only for very simple systems (such as an ideal gas), which allow the solution of the system configuration integral. In real systems, the computational determination of ΔG_{bind} can be carried out at different levels of approximation, with more accurate methods also being more demanding in terms of CPU time. A simple classification can be attempted according to the number of states that the system considers for the calculation (102). In path methods, the free energy difference is calculated by considering the initial and the final states together with several unphysical intermediates. The path in the energy surface connecting the unbound to the bound state is exceedingly long to calculate and the simulation would hardly converge. However, if the overall path is split into smaller steps, as in the free energy perturbation (103) or the thermodynamic integration (104) techniques, the local energy differences can be more practically calculated and total ΔG_{bind} can be obtained by summation. Other path methods currently used in binding free energy estimation are computational alchemy (105) and metadynamics (106). These methods are very accurate, providing ΔG_{bind} in the range of accuracy of the experimental error (1-2 kcal/mol), but their computational cost hampers their use in standard docking protocols. In the end-points methods, only the initial and the final states of the system are considered (93-107). The practical issue that has to be addressed here is that, in explicit solvent models, the free energy difference due to complex formation represents just a small fraction of the global energy difference between the two states, overwhelmed by solvent contributions. For this reason, many end-points methods resort to implicit solvent descriptions to highlight solute contributions to ΔG_{bind}. These strategies are much faster than path-based methods but still provide very accurate predictions. Several end-points methods have been successfully applied in docking protocols and one example will be discussed in greater detail later in this chapter.

In the vast majority of docking approaches, ΔG_{bind} is estimated using a simple scoring function that only considers the bound state (108). It has been noted that, for strong binders, this assumption can still provide an adequate description of the system because the bound conformation provides the main contribution to the partition function. Scoring functions are not expected to provide an accurate estimate of the binding free energy, but to characterize those igand poses that more accurately resemble experimental binding modes. To date, over 35 different scoring functions have been reported (43). They introduce different approximations and linearly combine different terms (which are generally assumed to be independent terms) but they all share the main feature of being fast: they can provide an almost instantaneous, if somewhat rough, estimation of the binding free energy. Scoring functions are also used *during* the sampling step, whenever an energy evaluation is necessary (application of the Metropolis criterion, estimation of the fitness function in GA, comparative conformers evaluation in incremental construction, etc.). So, technically, the final scoring step should be more properly referred to as re-scoring, since contributions to the free energy of binding ignored during sampling for the sake of speed can be included at this stage or an altogether different scoring function can be used. Several authors have classified the scoring functions into three main categories: force-field-based, empirical, and knowledge-based (16,30,109).

3.6.1. Force-field-based scoring functions

The bonded and non-bonded interactions among the atoms in the system are modeled according to the rules of molecular mechanics. A master equation provides the overall energy of the system, expressing different contributions with additive terms. The terms expressing the energy strain that the ligand displays in its bound conformation are described by a harmonic potential: the energy contribution of covalent bonds stretching, valence bonds bending, and torsions varies according to the deviation from a reference value. A Coulomb electrostatic potential describes the interactions between charges, while van der Waals attractive and repulsive energies are expressed by the Lennard-Jones potential. Equilibrium states for these terms are derived from force fields originally developed for molecular dynamics calculations. Since functional forms differ in just minor details among different force fields, only the equation of the AMBER (110) force field is reported as a general example:

Eq.3

$$E_{TOTAL} = \sum_{bonds} K_r(r - r_{eq})^2 + \sum_{angles} K_\theta(\theta - \theta_{eq})^2 + \sum_{dihedrals} \frac{V_n}{2}[1 + cos(n\phi - \gamma)] + \sum_{i<j}\left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}}\right]$$

Due to the nature of the resulting energy landscape, a minimization step is required before the final energy evaluation (109). The main limitation of force-field-based scoring functions is that contributions to binding such as desolvation effect and configurational entropy loss are either completely overlooked or introduced in the final score by heuristics. Several authors suggest that the accuracy of force-field-based scoring functions can be increased by tuning the Lennard Jones potential with different exponents (111). In fact, in its standard 6-12 implementation, this term is extremely sensitive to even small deviations in atomic coordinates and can produce a large amount of noise in intermolecular energy calculations. Another way to improve accuracy is to work on the electrostatic potential. Force fields that explicitly account for polarization effects on the atomic charges as well as distant-dependent dielectric constants to model solvation have recently been adopted in docking protocols (112), D-Score (111), G-Score (111), and the scoring function used in AutoDock (92) are all examples of force-field-based scoring functions.

3.6.2. Empirical scoring functions

Empirical scoring functions are apparently similar to force-field-based implementations as they are built on the idea that a binding score can be described through a linear summation of independent terms. Empirical terms may vary among scoring functions but they are usually simpler than their force field counterparts, can be calculated very easily, and are statistically weighted before summation. The weights are determined by regression analysis from a training set of known ligand receptor co-crystals whose binding free energy was experimentally calculated. What is not clearly established and is usually regarded as the main limitation of this kind of scoring method is the efficacy of their predictive power when confronted with putative ligand receptor complexes that are radically different from those used in the training set. Several empirical scoring functions have been reported, some developed and used in tight combination with a specific docking software (GlideScore in Glide (55), or the scoring functions used in ICM (113) and FlexX (45)), other provided as standalone routines, like ChemScore (114), HINT (115), and VALIDATE (116). A very appealing feature of empirical schemes is the possibility of devising customized weights in order to tailor the function toward a specific protein class. F-Score (45), one of the scoring functions endowed in FlexX and largely based on the work of Böhm (117), represents a standard example of the empirical approach. The binding score is calculated by combining simple terms that account for hydrogen bonds (hbond), ionic interactions (ionic), the number of torsional angles (N_{rot}), and protein-ligand lipophilic contacts (lipo).

Eq.4

$$\Delta G = K_0 + K_{rot}(N_{rot}) + K_{hb}\sum_{hbond} f(\Delta R, \Delta\alpha) + K_{ionic}\sum_{ionic} f(\Delta R, \Delta\alpha) + K_{aro}\sum_{aro} f(\Delta R, \Delta\alpha) + K_{lipo}\sum_{lipo} f(\Delta R)$$

The functional form $f(\Delta R,\Delta\alpha)$ of each term discourages deviations from ideal geometries. With respect to the previous work (118), the main novelty introduced in F-Score is the calculation of lipophilic interactions as a sum of all pairwise interatomic contacts. Moreover, the scheme introduces a specific term for aromatic contributions (aro). The K weights were calibrated by regression using a set of 45 experimentally determined binding scores from protein-ligand co-crystals.

3.6.3. Knowledge-based Scoring Functions

A third strategy that can be adopted to provide a quantitative assessment of the binding energy is related to the idea of Potential of Mean Force (PMF) (119). The PMF formalism was originally developed for liquids statistical mechanics and later adapted to proteins in folding-related studies (104). In proteins, an analysis of the frequencies of interatomic contacts is carried out on a training set of crystal structures: the most favorable interactions should be located in the maxima of the frequencies' distributions. The contribution to the binding free energy of each atom pair is calculated according to the collected statistics and the total binding score is generated through a summation over all the interactions. On average, knowledge-based scoring functions achieve satisfactory performances, they are very fast, and comparatively easy to implement. The results they provide are not greatly dependent on the nature of the training set, unlike the results of regression-based methods. However, knowledge-based scoring functions can lack physical rigor: in systems of equal particles in thermodynamic equilibrium, an n-particle correlation can be translated into a potential that gives an average force over all the configurations of the system. However, atoms in protein ligand complexes are not equal and a set of crystal structures cannot be considered a system in equilibrium. In 2000, Gohlke and colleagues developed and validated DrugScore, a knowledge-based scoring function that increased the accuracy of FlexX by up to 75% on a test set of over 150 proteins (120). DrugScore's main equation relates two different kinds of contributions: distance-dependent pair potentials between atoms of the ligand and atoms of the protein and a one-body potential term that accounts for the surfaces in the two molecules (SAS_0) that become buried upon complex formation (SAS). The preference ΔW for a specific ligand pose is expressed as:

Eq.5

$$\Delta W = \gamma \sum_{k_i} \sum_{l_j} \Delta W_{i,j}(r) + (1-\gamma)\, X \left[\sum_{k_i} \Delta W_i(SAS, SAS_0) + \sum_{l_j} \Delta W_j(SAS, SAS_0) \right]$$

considering k_i atoms of the ligand and l_j atoms of the protein.

The role of hydrogen atoms and the entropic contribution to the binding free energy are considered implicitly in this kind of calculation. Of several scoring functions that have been reported, SMoG2001 (121) and M-Score (122) are worth mentioning.

3.6.4. Consensus scoring

The consensus approach is a very straightforward attempt to overcome the limitations of the currently available scoring functions. Rather than a single method, scoring functions are combined to evaluate the generated poses (123-124). This approach is reported to significantly enhance the accuracy of the results. The consensus approach is unlikely to outperform the most accurate of the scoring functions used. However, in a real life scenario, it is not possible to know in advance which scoring scheme is going to perform better or worse than the others. By using several of them, the below-average performance of a particular scoring function is less likely to affect the overall quality of the results (125). Attempting a different strategy to address the scoring problem, we considered the poses' ensemble as an actual collection of observed data to be dealt with using a statistical approach (126-127). The application of advanced clustering techniques significantly improved the accuracy of the docking results, establishing a significant correlation between cluster population and the presence of a near-native pose.

4. COMPARING Docking Protocols

In 2010, over 70 docking engines are commercially or freely available to the scientific community, and new implementations are reported every month (43). Several groups (109,128,132) have attempted to compare the performances of different docking protocols, in terms of both sampling and scoring, to answer legitimate and compelling questions. Which docking tool works best? Do we really need so many implementations? Are new programs performing better than the old ones? Although no method appears to systematically outperform the others, researchers did identify combinations of sampling and scoring that perform better for a specific target or series of compounds. In this light, comparative reports can help select the docking approach most appropriate to the task at hand, and so interest in this kind of exercise remains high. However, setting up a truly fair comparison is far from simple for a number of reasons, discussed in two excellent reviews by Cole *et al.* (133) and, more recently, by Hawkins *et al.* (22). An indirect comparison based solely on the results obtained by each tool in the validation process would hardly provide robust conclusions: each tool was usually validated against a benchmark of specifically collected crystallographic complexes which only marginally and accidentally overlapped with those used for other programs. Furthermore, structures in each set were selected according to different criteria and the success rates were not estimated in a uniform way. In a direct comparison, different tools are tested against a purposely compiled set of co-crystals. Ideally, such a set should only include high quality structures to limit the chance of failure due to intrinsic biases of the crystals and not limitations of the docking approach. As mentioned, structures should also display a high level of diversity. Hartshorn and colleagues (21) reported specific guidelines for compiling a set of protein structures to validate and compare docking tools. According to these guidelines, they compiled a publicly available benchmark of 85 structures of pharmaceutical relevance. A fair comparison should also consider the user's knowledge of the docking protocols compared in the study as well as the user's familiarity with the systems included in the test set. Several studies reported a consistent improvement of docking accuracies when protocols were purposely adapted to the system, often deviating quite significantly from the set of default or suggested parameters (134). Similarly, the outcomes improved when the setup exploited expert knowledge of the system biology, i.e. manually assigning hystidines' tautomeric states or including crystallographic water molecules that greatly affect the binding event.

5. FUTURE PERSPECTIVES

Modern drug discovery is evolving and new approaches such as fragment-based drug design (135-136) and polypharmacology (137) are steadily becoming more and more popular. Can currently available docking schemes, developed to predict the binding mode of a lead-like molecule to the rigid binding site of a single target, efficiently assist these new paradigms? Preliminary evidence suggests that the available protocols will need significant updates and improvements but will not have to be rethought from scratch (138).

For example, during validation and fine tuning, traditional assessments of accuracy could be complemented by figures of merit solely based on ligand-receptor interactions, such as interaction fingerprints (139), that better describe the behavior of fragments with respect to RMSD. Scoring functions will have to be recalibrated to accurately predict the binding mode of weak binders, since fragments tend to display experimental K_i values in the high micromolar range (140). Again, the entropic contributions to the binding energy will have to be calculated much more accurately since rough approximations proportional to the number of torsional degrees of freedom work acceptably for lead-like compounds but would dramatically fail in simulations involving low affinity fragments.

Polypharmacology, namely the ability of a compound to modulate multiple targets at the same time, is emerging as the leading strategy for interacting with complex pathologies, overcoming back-up and redundant mechanisms in a disease network (141). A truly efficient and systematic implementation of docking in multi-target strategies will require a simultaneous treatment of different binding sites, most likely exploiting procedures usually used in pharmacophore search and ligand-based strategies (142-143). The preliminary results obtained by docking simulations in the field of multi-target ligand development suggest that this technique will be a valuable tool in future research. For example, Chronic Myelogenous Leukemia (CML) is an aggressive neoplasy characterized by an unregulated overgrowth of the myeloid cells in the bone marrow. It is a good example of a complex disease (144). CML is triggered by a reciprocal translocation in chromosomes 9 and 22, resulting in an aberrant chromosomic structure known as Philadelphia; the Philadelphia translocation creates Bcr-Abl, an oncogenic fusion gene translated into a constitutively active tyrosine kinase domain. The kinase activity deeply affects several cell cycle regulators, boosting myeloid cells' proliferation rate. CML is currently treated with tyrosine kinase inhibitors such as Imatinib (145). However, the emergence of Imatinib-resistant tumor clones in patients treated with Bcr-Abl kinase inhibitors led to the development of novel molecules that could interact with several variants of Bcr-Abl or with Bcr-Abl and other targets(144). Ligand docking has been used to identify dual inhibitors of Bcr-Abl and Src, a member of a proto-oncogenic tyrosine kinase family that emerged as an ideal co-target, since it is overexpressed in leukemia cells and participates in CML development (146). Manetti *et al.* reported a lead discovery protocol built on a combination of molecular dynamics simulations and docking studies that led to the development of dual c-Src/Abl kinases inhibitors (147). The same enzyme combination was also targeted by a series of pyrazolopyrimidines designed using a consensus application of two different docking engines.

Ligand docking applications in multi-target ligand identification is not limited to molecules used to treat CML: in combination with pharmacophore matching, it was also a key step in identifying three dual inhibitors that target human leukotriene A_4 hydrolase (LTA4H-h) and the human nonpancreatic secretory phospholipase A2 (hnps-PLA2) (148). Both enzymes are involved in the arachidonic acid metabolism and a concurrent inhibition of the same pathway in two different spots is considered a promising strategy in treating inflammation. Jenwitheesuk and colleagues reported the use of docking in combination with other computational techniques to develop molecules active against HIV-1 retrovirus and, at the same time, other pathogens responsible for opportunistic infections (149). Although the multi-target profile is limited here to very closely related enzymes or mutants of the same target, the reported studies clearly show that ligand docking can beneficially assist in the discovery of multi-target ligands.

6. CONCLUSIONS

Over the last 30 years, the reliability of docking protocols has improved constantly, to the point where the most recent implementations address quite efficiently some classic shortcomings of the technique. Several issues remain, primarily the accuracy of binding energy predictions. But important breakthroughs are expected thanks to the ever-increasing computational power of multicore CPUs (150). Presently, ligand docking plays an important role, especially in the hit-to-lead phase of drug discovery projects where it helps rationalize SAR data and design novel decorations (151). Moreover, thanks to the variety of docking software available, personalized protocols can be devised for specific targets or specific ligand-target combinations. In summary, ligand docking is now a valuable part of almost every structure-based drug design study carried out in both academia and industry.

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Abbreviations: CADD: Computer-Assisted Drug Design; LBDD: Ligand-Based Drug Design; SBDD: Structure-based Drug Design; PDB: Protein Data Bank; GA: Genetic Algorithm; MC: Monte Carlo; MD: Molecular Dynamics; MRC: Multiple Receptor Conformations; RCM: Relaxed Complex Scheme; MM-PBSA: Molecular Mechanics Posisson Boltzmann Surface Area; RMSD: Root Mean Square Deviation; PMF: Potential of Mean Force.

Key Words: Docking, structure-based drug design, receptor, ligand docking, protein docking, ensemble docking, receptor flexibility, cross docking, MRC, scoring function, consensus scoring, review.

Send correspondence to: Giovanni Bottegoni, Dept. of Drug Discovery and Development, Istituto Italiano di Tecnologia, Address: via Morego n.30 16163 Genova, Italy, Tel: 39 010 71781522, Fax: 39 010 7170187, E-mail: giovanni.bottegoni@iit.it

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