PART I

COMPUTATIONAL AND STRUCTURAL APPROACHES IN DRUG DISCOVERY

CHAPTER 1

MOLECULAR DOCKING AND STRUCTURE-BASED DESIGN

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1.1 INTRODUCTION

The discovery of new drugs is a complex process. It generally starts with the identification of compounds that bind to a target or show efficacy in a simple screen. Molecules that show good affinity are called ''hits.'' The next step is to find compounds that have attractive pharmaceutical properties—for example, low toxicity and sufficient aqueous solubility to be orally active. Such compounds are often called ''leads.'' Traditionally, ''hits'' have been found by screening, while ''leads'' are developed from ''hits'' through chemical synthesis. Screening normally involves large numbers of compounds from natural products, corporate databases, or organic chemistry companies that can be examined for biological activity in high-throughput assays. Commercial systems can process millions of tests per day for enzyme targets. The best compounds are moved forward in a process aimed at modifying their chemical structure to improve potency, specificity, and *in vivo* activity while lowering toxicity and side effects. Synthetic methods include combinatorial chemistry and library synthesis (Fig. 1.1).

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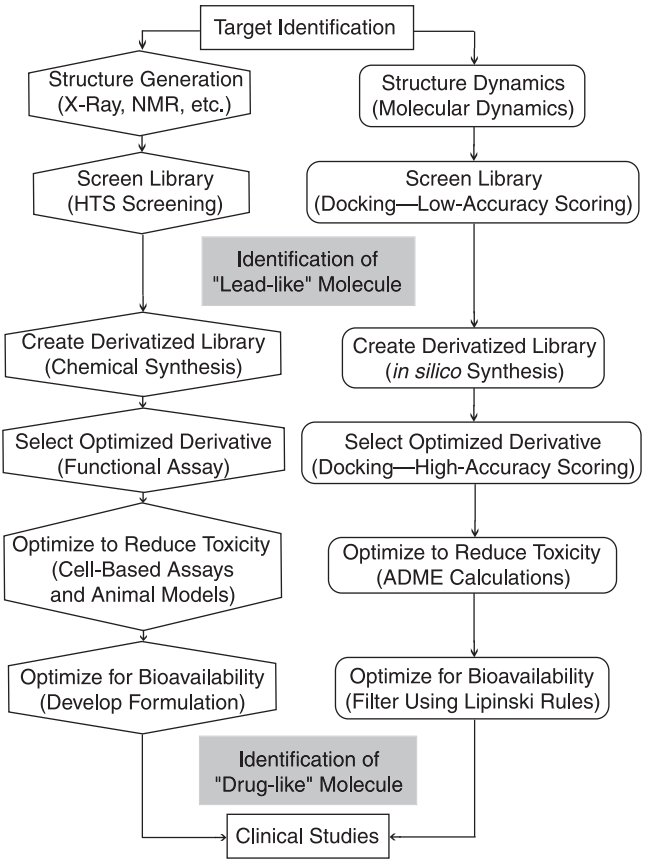


Figure 1.1 Example flow of drug design process from both experimental (left) and computational (right) perspectives.

Computational methods have proved useful in many aspects of the discovery process (Alvarez, 2004). A variety of strategies are available. If an active ''lead'' is known, it is straightforward to query a database for molecules with similar properties using pharmacophore searches or quantitative structure–activity relationships (QSAR). These methods typically use information available for the ligand, inhibitor, or substrate rather than the receptor. However, there is growing interest in mapping out receptor properties—either from known family relationships with other members of the receptor family or through pharmacophore strategies applied directly to the receptor structures (Arnold et al., 2004; Hajduk et al., 2005). This chapter focuses on a set of strategies in which direct knowledge of the receptor structure is used to identify or design ligands that possess good steric and chemical complementarity to specific sites on the target macromolecule. This process is referred to as ''structure-based drug design'' (Brooijmans and Kuntz, 2003).

The structure-based drug design paradigm is analogous to experimental screening. Structures for the receptor or target are obtained either from the literature or from in-house operations. These structures come from crystallography or nuclear magnetic

resonance (NMR) experiments, but there is increasing interest in high-quality homology-modeled structures (Chance et al., 2004). Computer analogs of ligands are generated. These families are often called ''virtual libraries'' and may consist of compounds from corporate, academic, or commercial holdings (Laird and Blake, 2004; Webb, 2005). A virtual library might also include molecules that are not physically available but might be obtained through chemical synthesis, perhaps using combinatorial chemistry (Jorgensen, 2004; Kick et al., 1997). Screening of the virtual library against the target structure involves some form of positioning the putative ligand in three-dimensional space and evaluating the intermolecular interactions for that particular geometry. Typically, the process is an iterative one: The ligand is moved, and the new geometry is evaluated. This cycle is repeated until some ''best scoring'' geometry is identified for the particular ligand under test. Then, the next ligand in the list is chosen, and the whole procedure begins again. The goal of virtual screening is to identify the best binding candidates from the library for experimental testing. Because the virtual libraries can be huge—upwards of a billion compounds—this triage procedure is a critical step.

Once a binding candidate has been found, structure-based design can be used to optimize binding affinity. For this operation, one starts with a ''hit'' or ''lead'' with a known activity. Often, a structure for the ligand–target complex is available. There are many computational methods available for evaluating chemical variants of the ''lead'' that offer suggestions about the direction for the next round of synthesis. Ideally, a number of such variants are prepared, and their properties and structures are obtained so that a selection of molecules are available to take into further biological testing. Optimization methods are typically much more computationally intensive than other virtual screening approaches.

There has recently been a merging of these two ideas. Virtual libraries containing 1000–10,000 molecular fragments (sometimes called ''anchors'' or ''scaffolds'') are used in the initial screening. The most promising are then expanded using computer synthesis in a combinatorial fashion (Jorgensen, 2004; Kick et al., 1997; Miranker and Karplus, 1991).

In this chapter, we will focus on a particular subset of molecular design strategies called ''docking,'' in which candidate molecules are matched to receptor structures and evaluated for chemical and geometric complementarity. We will not discuss the broad field of quantitative structure–activity relationships (QSAR) that focus on the chemical structures of ligands alone (Bender and Glen, 2005).

1.2 MOLECULAR DOCKING

1.2.1 Overview

The basis of molecular docking is the calculation or estimation of the free energy of binding of a ligand to a specific receptor site in a fixed environment. The free energy of binding yields, directly, an equilibrium binding constant and, indirectly, the preferred binding mode of the ligand–receptor complex. There are important scientific and mathematical issues involved. For example, it is currently much easier to calculate the energy/enthalpy of interaction than to obtain the free energy because we lack efficient

ways to obtain the entropic contributions. Second, the interactions of the ligand and receptor with the solvent (water, salts, and other components) are not easy to quantitate. Third, searching through the large number of conformations of the receptor and the ligand and their relative positions are difficult computer science problems. The need to repeat these calculations for large numbers of putative ligands and many possible targets requires serious attention to the algorithms. Docking protocols have adopted a variety of heuristics to make useful calculations with the knowledge that a complete high-level calculation is not feasible for the systems of biological or therapeutic interest.

What can we expect from current approaches? The best-case calculations are accurate to within approximately 0.5 kcal/mol of experimental results, but these are generally free energy differences obtained using perturbation techniques on a related family of ligands (Jorgensen, 2004). Routine results are rarely within 1 kcal/mol of experimental results, and library searches of diverse chemical types have larger inaccuracies. Work continues on improving the force fields that model the enthalpic terms (Bernacki et al., 2005). Estimates of entropic contributions are empirical, and adequate sampling of configurations and conformations search is a complex combinatorial problem. Consequently, searching large databases for new leads requires protocols that deal with four specific tasks:

1. Receptor site identification
2. Receptor site characterization
3. Orientation of the ligand within the site
4. Evaluation of the ligand

These steps are described in turn in the succeeding sections, and examples are given.

1.2.2 Receptor Site Identification

With the sequencing of the human genome and recent advances in structural techniques, the number of publicly available biomolecular structures has exploded over the last few years with over 40,000 in the Protein Data Bank (Berman et al., 2000). The two main experimental sources for three-dimensional (3D) structures of biomolecules are X-ray crystallography and high-resolution NMR spectroscopy. X-ray crystallography provides structures of biomolecules in the crystalline states, and with the crystallization of the ribosome, the upper limit of the experiment has been extended to the 1000-kDa range. NMR spectroscopy is limited to approximately 50 kDa, but the method has the advantage of providing additional information about the dynamics of the structure. As an alternative, if the structure has not been solved experimentally, computational techniques such as homology modeling can be used to predict 3D structures. We next discuss some pros and cons of each of these sources for structure-based drug design.

For X-ray crystal structures to be sufficiently accurate for drug designing purposes, a resolution of approximately 2 Å, an R-factor below 20%, and an R_{free} factor below 30% are preferred. It is important to note that the majority of 3D crystal

structures of biomolecules do not have hydrogens or highly flexible residues included in the file. The missing atoms must be considered before structure-based drug design can begin. In addition, crystal packing forces may locally influence protein conformation, particularly for nucleic acids and surface active sites.

The result of structure determination with NMR is an ensemble of structures that agree equally well with experimental data. Although an averaged structure can be derived, it has been shown that the entire ensemble provides a more complete description of the system from an experimental perspective (Staunton et al., 2003). For structure-based drug design purposes, though, there are several methods for choosing the appropriate structure, including selecting the member of the ensemble closest to the average as measured by some distance metric or cross-docking to all members of the ensemble. Unfortunately, there is no generally accepted standard of accuracy for NMR-generated structures. As a rough rule of thumb, a high-resolution NMR structure should preferably have approximately 20 (distance or dihedral) restraints per residue (Berman and Westbrook, 2004).

If no experimental structural information is available for the target biomolecule, homology modeling can provide structures to guide the search for novel lead compounds. It should be noted that, depending on the method, homology modeling yields average errors of 3-Å root-mean-square deviation of proteins with greater than 50% sequence similarity, with larger errors for increasing sequence dissimilarity (Nissen et al., 2000). Nevertheless, homology modeling has proven to be successful in several cases, including discoveries of a highly potent DNA methylation inhibitor and a compound that discriminates between two voltage-gated K^+ channels with 20-fold accuracy (Staunton et al., 2003).

A number of more general issues associated with the selection and preparation of a receptor structure should be noted. In many structures, ions are required for structural or functional purposes. However, modeling this type of chemistry is often difficult because the formal charge and associated desolvation energy of ions are extremely complicated to compute accurately. The protonation states of residues such as histidine, lysine, glutamic acid, or aspartic acid are highly dependent on the environment in the active site and may even change in response to the ligand (Hensen et al., 2004). In particular for crystal structures, critical water molecules may be present in the active site, and it is often difficult to predict whether the water can be replaced or should be included in the model of the receptor. All these issues can affect the quality of the model and should be carefully considered.

1.2.3 Receptor Site Characterization

Once an accurate structure has been determined for the target, ligands are typically restricted to lie within one geometric region of the macromolecule, generally known as the ''binding site.'' This region is generally selected because, upon ligand binding, normal function is altered. A given receptor can have one or more binding sites, such as the active sites of enzymes, allosteric sites, the binding or recognition sites of receptors, or even a dimer interface. The exact location of the binding site may be well known through experiment. However, if the binding site is not known, automated methods exist to identify potentially interesting regions.

Experimental data that indicate the binding site is the best source, if available. Experimentally derived structures of the biomolecule complexed with the natural substrate or a known inhibitor directly indicate the binding site. The Protein Data Bank and the Nucleic Acid Data Bank contain a large number of these types of structures (Berman et al., 1992, 2000). The Cambridge Crystallographic Data Centre/Astex has compiled a subset of biologically relevant protein–ligand complexes identified as being reliable for structure-based drug design purposes (Nissink et al., 2002). If direct observations are not available, binding regions can be identified through mutational experiments (such as alanine screening) or other biochemical assays.

There are cases where binding site information is not available. In these instances, computational tools can be used to indicate probable binding areas. We will describe two methods: The first is based upon geometric features, and the second uses chemical functionality of the receptor surface. For illustration, we consider SPHGEN from the DOCK suite of programs and the Multiple Copy Simultaneous Search (MCSS) approach (Ewing et al., 2001; Kuntz et al., 1982; Meng et al., 1992; Miranker and Karplus, 1991). In addition, an interesting statistical characterization has been recently described (Hajduk et al., 2005).

SPHGEN automatically identifies a target site by computing a set of site points or sphere centers, which serves to create a negative image of the surface. The algorithm begins by mapping the geometric features of the receptor surface, as defined by Lee and Richards, using the dms program (Richards, 1977). Then, spheres of varying radii are analytically generated to touch the molecular surface at two points, with the sphere center lying along the surface normal and with no portion of the sphere intersecting with a receptor atom. These overlapped spheres indicate various surface features, including invaginations and clefts. A clustering protocol, using radial overlap as a metric, is then used to indicate potential areas for ligand binding. The largest cluster is generally used as the binding site, and, once generated, the cluster is used as a template for possible ligand atom positions.

MCSS identifies binding sites by mapping the chemical properties of the biomolecular surface. Thousands of copies of specific molecular fragments are distributed in the target region of the protein. Then, energy minimization is performed on the ensemble, creating distinct local minima for each fragment. This process is repeated on a variety of chemical functionalities until the surface is adequately described. Although this method does not capture every geometric detail of the binding site, it does provide a basic pharmacophore that can be used in later studies (Arnold et al., 2004; Miranker and Karplus, 1991).

1.2.4 Orientation of the Ligand in the Target Site

There are two basic strategies for exploring the orientational degrees of freedom for putative ligands. The first uses a search grid for both the translational space and the euler angle space. This brute-force method is feasible if one is studying a few ligands in a restricted site. It can be extended to larger libraries if parallel processing is available (Jorgensen, 2004). Alternatively, protocols have been developed to pre-screen orientation space. For example, the DOCK program uses a geometric pairing

algorithm, matching the sphere centers (described above) with ligand centers (usually ligand atoms). The matching criterion is based on a comparison of intersphere and interatom distances. Exhaustive or selective searches can be done over the match matrix. Careful placement of the spheres is an important step in getting good-quality results. The second type of method selectively samples all of orientation and conformation space using search engines. For example, the Metropolis algorithm and simulated annealing are used in QXP, and the genetic algorithm has been implemented an Autodock (McMartin and Bohacek, 1997; Morris et al., 1998). These algorithms have been studied extensively for many applications. Their strengths and limitations are well understood.

1.2.5 Evaluation of Ligand Orientations

The many configurations (orientations and conformations) of the ligand need to be evaluated with a scoring function to identify the energetically most favorable ligand binding pose. Ideally, the scoring function would calculate the ligand free energy of binding in aqueous solution (Beveridge and Dicapua, 1989; Kollman, 1993). However, the large computational expense of these calculations leads to the introduction of scoring functions that calculate a range of simplifications of the ligand binding free energy.

Scoring functions can be broadly classified into two categories: those based on first-principles-derived molecular mechanics force fields, and those based on functions fit to empirically derived binding data. For the purposes of this review, scoring functions that employ quantum mechanics are not considered as the extreme computational cost of these calculations make them prohibitive for use during small-molecule docking.

Of the first-principles-derived scoring functions, the most computationally efficient are those that approximate the binding free energy (ΔG_{bind}) as the molecular mechanics protein–ligand interaction energy. Molecular mechanics treats the molecule as a collection of atoms governed by a set of classical mechanical potential functions (Weiner and Kollman, 1981). Parameters for these potentials are derived from small-molecule experiments and refined to yield correct structural and thermodynamic quantities such as bond stretching frequencies or heats of formation. The primary DOCK energy scoring function approximates the ligand–receptor binding energy using the AMBER molecular mechanics intermolecular interaction energy, a sum of the Lennard-Jones 6–12 van der Waals (vdW) potential, and the Coulombic potential, given in equation (1.1):

$$E = \sum_{i}^{\text{Lig}} \sum_{j}^{\text{Rec}} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} + K \frac{q_i q_j}{D r_{ij}} \right) \tag{1.1}$$

where i indexes the ligand atoms and j indexes the receptor atoms; A and B are the vdW attractive and dispersive parameters, respectively; q is the partial charge on the atom; K is the scaling constant that converts electrostatic energy into kcal/mol; D is the dielectric constant of the medium; and r_{ij} is the distance between ligand atom i and receptor atom j (Pearlman et al., 1995). This scoring function is limited by its use of a distance-dependent dielectric screening function to mediate all charge–charge

interactions. This dielectric treatment assumes that the dielectric value of the solvent is uniform between all charge pairs.

A class of scoring functions has been developed that utilizes implicit models of solvation to calculate the electrostatic component of the molecular mechanics intermolecular interaction energy in a more sophisticated fashion than the simple Coulombic approach previously described. Both the Generalized Born (GB) and Poisson–Boltzmann (PB) terms have been combined with an empirically derived surface area (SA) term to include the energy of desolvating nonpolar atoms, and the resulting GB/SA and PB/SA methods have been implemented into molecular dynamics and docking methods (Feig and Brooks, 2004; Feig et al., 2004; Honig and Nicholls, 1995). While these functions are more computationally intensive than the Coulombic electrostatic energy functions, their proper treatment of solvation effects yields more accurate energy scores, and they are therefore frequently used in a hierarchical fashion to rescore docked ligand poses. DOCK 5 implements a GB/SA scoring function that is recommended for use in a rescoring capacity (Zou et al., 1999).

The most computationally intensive class of first-principles-derived scoring functions combine molecular dynamics (MD) simulations with implicit or explicit solvation to average the interaction energies from a Boltzmann-weighted ensemble of complex structures, yielding accurate estimates of the binding free energy that takes into account protein flexibility. The implicit solvation methods, MM-PB/SA and MM-GB/SA, perform a short, explicit solvent MD simulation from which a set of snapshots of the protein–ligand complex structure are saved (Gohlke and Case, 2004; Wang et al., 2001; Zhou and Madura, 2004). These snapshots, representing a Boltzmann-weighted ensemble of complex structures, are rescored with either the PB/SA or GB/SA scoring functions, and the average interaction score of the snapshots is taken as the free energy of binding for the ligand. Several research groups in academia and industry use these methods in a hierarchical fashion to rescore ligands identified as potential binders (Kollman et al., 2000).

The second major class of scoring functions models the binding free energy as a weighted sum of several different types of interaction energies, with and without explicit vdW and electrostatic terms. Many of these functions are based upon a comparison of receptor–ligand complexes and experimental binding data (K_i). Programs such as AutoDock, FlexX, GOLD, and Glide implement a variety of empirically derived energy score functions, including the well-known ChemScore and PLP functions (Eldridge et al., 1997; Friesner et al., 2004; Gehlhaar et al., 1995; Halgren et al., 2004; Hoffmann et al., 1999; Morris et al., 1998; Verdonk et al., 2003).

1.3 LIGAND STRUCTURE GENERATION

To identify virtual screening "hits," databases consisting of three-dimensional structures of putative ligands are searched using the methodology described above. How are these structures obtained? In some cases, structures can be taken directly from a database of experimentally determined structures; for example, the Cambridge Crystallographic Database contains over 360,000 small-molecule

crystallographically determined structures. In cases where only the two-dimensional information is known, one can create computer representations of the covalent structure using conformation generation programs such as Concord (Tripos), ChemX (Accelrys), Rubicon (Daylight), and Omega (OpenEye). These programs provide one or more conformers consistent with the chemical connectivity and general rules of physical organic chemistry. Often, these conformers will be ranked by some energy formula. Finally, some vendors make libraries of three-dimensional structures directly available, including the Advanced Chemical Development (www.acdlabs.com), MDL Drug Data Report (www.mdl.com), National Cancer Institute Open Database Compounds (cactus.nci.nih.gov), Tripos Discovery Research Screening Libraries (www.tripos.com), InfoChem GmbH database (www.infochem.de), Thomson Index Chemicus database (scientific.thomson.com), and ZINC (blaster.docking.org/zinc).

Two strategies have emerged to study flexible ligands. The first, generally referred to as incremental construction, breaks down the ligand into smaller pieces and then rebuilds it during the docking calculation. One example of this technique starts with a fragment of the compound (an ''anchor'') and then adds atoms in layers during a docking or an optimization cycle. This approach has been called ''anchor and grow.'' In the alternate method, conformers for each compound can be pregenerated, stored in a database, and then rigidly docked. Molecular dynamics and Monte Carlo techniques offer a combination strategy where the starting point is a single conformation of the ligand that then explores alternatives during the dynamics phase. There have been some tests of the two strategies, but there is no strong consensus of which is better (Lorber and Shoichet, 2005; Moustakas et al., 2006).

Other important issues that influence ligand structure and docking are choices of partial charges, tautomer preferences, and pK_a values. A new database (ZINC) that deals with many of these concerns is now available through the Shoichet group at UCSF (Irwin and Shoichet, 2005).

1.4 DESCRIPTION OF DOCKING PROGRAMS

While we do not have the space to provide descriptions of the many different approaches to molecular docking, Table 1.1 summarizes features of several of the most frequently used programs. We also present a brief synopsis of DOCK, developed at UCSF.

The current version of the DOCK program, is written in C++ and provides an object-oriented implementation in which each major component of the DOCK algorithm is a class with a documented interface, allowing these DOCK functions to be modified or replaced easily. As a result, it has been possible to independently validate and optimize the rigid body sampling, the flexible sampling, the energy scoring functions, and our minimizers. DOCK features an energy scoring function based on a molecular mechanics force field, solvation corrections using implicit solvent models, integration with the complete AMBER force field score, rigid body docking, ligand conformational searching, binding pose cluster analysis, and local minimization methods and also includes support for parallel computing using the MPI standard.

TABLE 1.1 Examples of Commonly Used Structure-Based Drug Design Packages

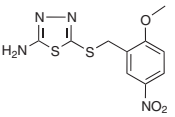
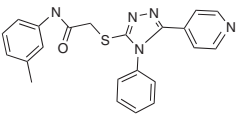
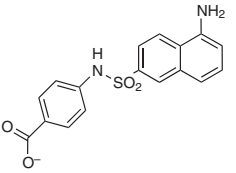
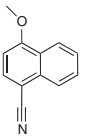
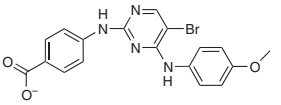
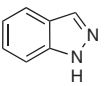
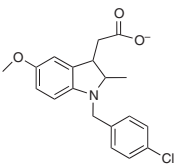
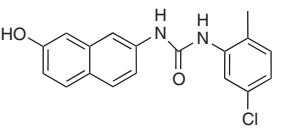
Method	Ligand Sampling Method[a]	Receptor Sampling Method[a,b]	Scoring Function[b,c]	Solvation Scoring[b,d]	Reference(s)
AutoDock	GA	SE	MM + ED	DDD, DS	Morris et al., 1998; Osterberg et al., 2002
DOCK 3	CE	SE	MM	PBE, DS	Lorber & Shoichet, 1998; Wei et al., 2004
DOCK 4/5	IC	SE	MM	DDD, GB, PB	Ewing et al., 2001; Knegtel et al., 1997 Moustakas et al., 2006
EUDOCK	CE	CE	MM	DDD	Pang et al., 2001a
FlexX/FlexE	IC	SE	ED	NA	Claussen et al., 2001
Glide	CE + MC	TS	MM + ED	DS	Eldridge et al., 1997; Friesner et al., 2004; Halgren et al., 2004; Sherman et al., 1996
GOLD	GA	NA	MM + ED	NA	Jones et al., 1997; Verdonk et al., 2003
ICM-Dock	MC	MC	MM + ED	DDD, PBE, DS	Abagyan et al., 1994; Totrov & Abagyan, 1997
MM-PBSA	MD	MD	MM	GB, PB	Kollman et al., 2000
QXP	TS + MC	MD	MM + ED	DDD	McMartin & Bohacek, 1997

[a]Sampling methods are defined as Genetic Algorithm (GA), Conformational Expansion (CE), Monte Carlo (MC), Molecular Dynamics (MD), Incremental Construction (IC), Merged Target Structure Ensemble (SE), and Torsional Search (TS); Section 1.3 for more information.
[b]If the package does not accommodate this option, the symbol NA (not available) is used.
[c]Scoring functions are defined as either empirically derived (ED) or based on molecule mechanics (MM); see Section 1.2.5 for more information.
[d]Additional accuracy can be added to the scoring function using implicit solvent models. The most commonly used options are Distance-Dependent Dielectric (DDD), Poisson Boltzmann Dielectric (PBE), a parameterized desolvation term (DS), Generalized Born (GB), and linearized Poisson Boltzmann (PB); see Section 1.2.5 for more information.

1.5 TESTS OF DOCKING AND STRUCTURE-BASED DESIGN

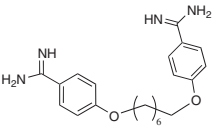
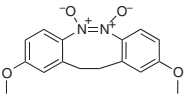
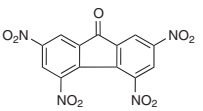
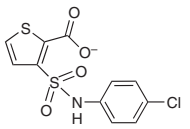
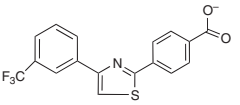
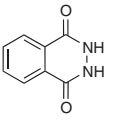
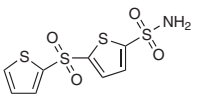
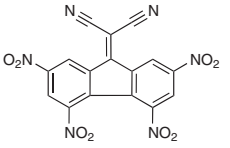
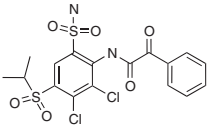
Despite well-known methodological weaknesses, structure-based screening using molecular docking has had important successes. Pragmatically, docking has suggested new, nonobvious ligands for multiple targets; these have been subsequently tested and shown to bind experimentally. Hugo Kubinyi, in a recent review, describes over 50 macromolecular targets for which ligands have been discovered using docking-based approaches (see Table 1.2 for a partial list) (Kubinyi, 2006). Most of these projects used experimental X-ray structures to represent the protein. In several cases, homology-modeled structures were employed (Evers and Klebe, 2004; Schapira et al., 2003).

TABLE 1.2 Recent Examples of Novel Inhibitor Discovery Using Molecular Docking

Target	Representative Hit	Lead Inhibitor IC_{50}	Follow-Up Inhibitor IC_{50}	Complex Structure
p56 Lck SH2 domain (Huang et al., 2004)		10 μM	NR	No
Neurokinin-1 receptor (Evers and Klebe, 2004)		0.25 μM	NR	No
AICAR transformylase (Li et al., 2004)		0.15 μM	NR	No
IMPDH (Pickett et al., 2003)		31 μM	NR	No
Checkpoint kinase 1 (Lyne et al., 2004)		0.11 μM	NR	No
DNA gyrase (Boehm et al., 2001)		10,000 μM	0 μM	Yes
Aldose reductase (Iwata et al., 2001)		4.3 μM	0 μM	No
CDK4 (Honma et al., 2001)		44 μM	0 μM	Yes

(Continued)

TABLE 1.2 *(Continued)*

Target	Representative Hit	Lead Inhibitor IC_{50}	Follow-Up Inhibitor IC_{50}	Complex Structure
Matriptase (Enyedy et al., 2001)		0.92 μM	0 μM	No
Bcl-2 (Enyedy et al., 2001)		10.4 μM	NR	No
Adenovirus protease (Abarbanel 1984; Pang et al., 2001b)		3.1 μM	NR	No
AmpC β-lactamase (Powers et al., 2002)		26 μM	1 μM	Yes
retinoic acid receptor (Schapira et al., 2001)		2 μM	NR	No
TGT (Gradler et al., 2001)		8.3 μM	0 μM	Yes
carbonic anhydrase II (Gruneberg et al., 2001)		0.0008 μM	NR	Yes
HPRTase (Freymann et al., 2000)		2.2 μM	NR	No
dihydro-dipicolinate (Paiva et al., 2001)		7.2 μM	NR	No

In recent work, the structures of known ligands in complex with their receptors have been predicted by docking, beginning with the structures of the independent molecules (Fig. 1.2) (Rizzo et al., 2000; Rosenfeld et al., 2003). In these studies, where the binding affinity is known but the structure of the complex is not, the docking predictions have been relatively accurate. The caveat to this is that there are many cases where docking mispredicts geometries in retrospective tests. Still, in published cases where the goal was genuine prediction, the docked geometry has often turned out to correspond closely to the subsequent experimental result.

A more difficult test is comparing the predicted geometries of novel ligands that emerge from the docking screens themselves. There are many examples of such predictions of ligand and geometry from docking screens against simple model cavity sites. These sites are small, completely enclosed by the protein, and dominated by one particular type of interaction, such as hydrophobicity, a single hydrogen bond acceptor, or a single electrostatic interaction. These features have allowed for multiple predictions of new ligands that are tested experimentally, often including structure

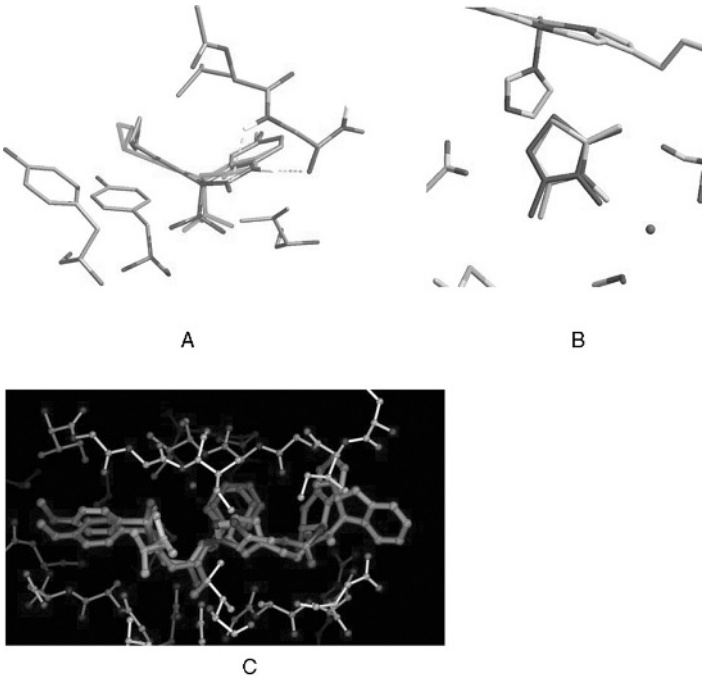


Figure 1.2 Predicted complexes versus X-ray crystallographic structures that were subsequently determined. (**A**) Predicted (carbons in gray) and experimental (green) structures for sustiva in HIV reverse transcriptase (Rizzo et al., 2000; Rosenfeld et al., 2003). (**B**) Predicted (magenta) and experimental (white carbon atoms) structures of 2,3,4-trimethylthizole in the W191G cavity of cytochrome c peroxidase (Rizzo et al., 2000; Rosenfeld et al., 2003). (**C**) Predicted (green) and experimental structure (carbons in gray) of an HIV protease inhibitor (ligands with thick bonds, enzyme residues with thin bonds) (Brik et al., 2003, 2005; courtesy of Art Olson, TSRI). See color plates.

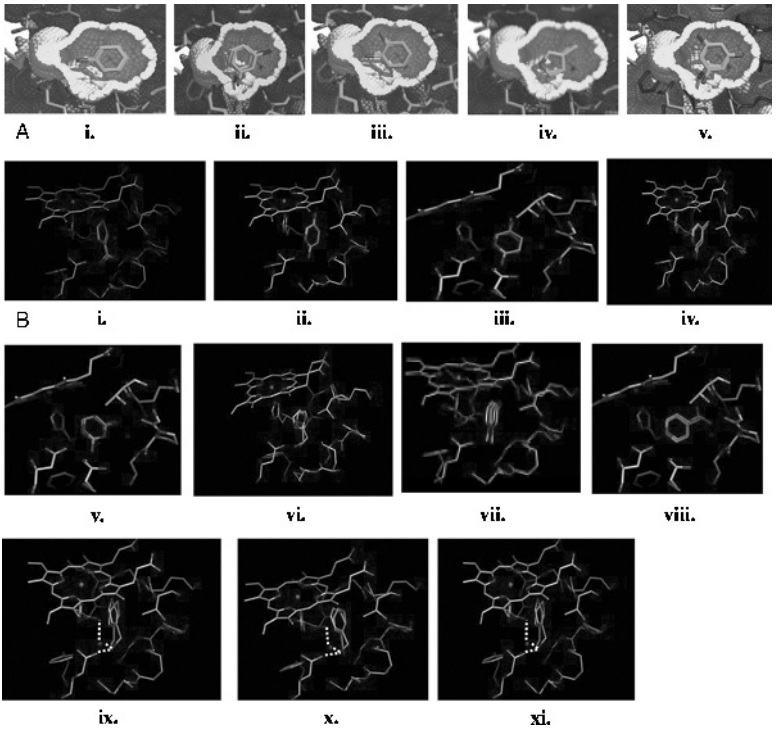


Figure 1.3 Docking predicted ligands from virtual screening against simple cavity sites. (**A**) The docked prediction (carbons in green) superposed on the crystallographic result (carbons in cyan). The surface of the L99A/M102Q cavity of T4 lysozyme (yellow) is cut away to reveal the complex. (i) Phenol, (ii) Chlorophenol, (iii) fluoroaniline, (iv) methylpyrrole, (v) difluorophenol. (**B**) The docked prediction (carbons in green) superposed on the crystallographic result (carbons in yellow) in the W191G cavity of cytochrome c peroxidase. (i) Thiophene-amidine, (ii) diaminopyridine, (iii) 2-amino-5-methylpyridine, (iv) 2-amino-4-methylpyridine, (v) diaminopyrimidine, (vi) hydroxymethyl-imidazole, (vii) 3-methyl-*n*-methylpyridine, (viii) 4-hydroxymethly-pyridine, (ix) aminomethyl-cyclopentane, (x) aminomethyl-benzene, (xi) aminomethyl-furan. See color plates.

determination (Fig. 1.3) (Brenk et al., 2006; Graves et al., 2005; Wei et al., 2002, 2004). Thus, X-ray crystal structures have been determined for about 25 ligands bound to three different cavities; in every case, the docking prediction corresponds closely to the X-ray crystallographic result. These results suggest that current docking algorithms are adequate to capture first-order determinants of binding fidelity (Fig. 1.4) (Gradler et al., 2001; Gruneberg et al., 2002; Powers et al., 2002; Wei et al., 2002).

How does performance in simple sites translate into larger, more drug-like sites? The consensus of many retrospective and prospective docking screens is that the ability to predict ligands and their geometries diminishes considerably in biology-relevant targets. In most cases, this failure reflects the increased complexity of the binding sites and the greater opportunities to find decoy ligand geometries. Nevertheless, there are examples of successful ligand prediction followed by structural

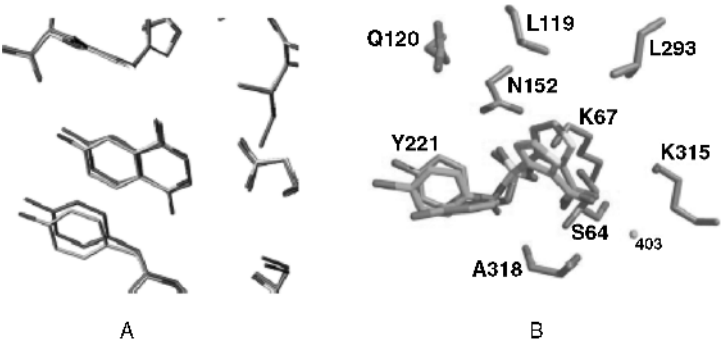


Figure 1.4 Predicted versus experimental structures from virtual screening. (**A**) The docked (carbons in orange) versus the crystallographic structure of the 4- aminophthalhydrazide bound to tRNA guanine transglycosylase (Gradler et al., 2001). (**B**) The docked (carbons in green) versus the crystallographic structure (carbons in orange) of 3-((4-chloroanilino)-sulfonyl)-thiophene-2-carboxylate bound to β-lactamase (enzyme carbons in gray) (Powers et al., 2002). See color plates.

determination, and in these cases the docking prediction is often close to the experimental result (Fig. 1.4) (Gradler et al., 2001; Gruneberg et al., 2002; Powers et al., 2002; Wei et al., 2002). These studies suggest that when the method does correctly predict a new ligand, even for a complicated, drug-like binding site, it does so for the right reasons.

An important question is whether structure-based screening is worth the effort, assuming that groups have access to high-throughput screening for ligand discovery. The two types of screens have been compared only a few times publicly, though rather more in unpublished industrial work. In the few published studies, the virtual screens had ''hit rates'' 10- to 1700-fold higher than the empirical screens (Table 1.3) (Doman et al., 2002; Kick et al., 1997; Oshiro et al., 2004; Paiva et al., 2001; Wyss et al., 2003). In the case with the best hit-rate enhancement, that of the diabetes-associated enzyme PTP-1B, the comparison was an imperfect one. Here, different libraries were targeted by the virtual and high-throughput screen, and a slightly different assay was used. In very recent work, Eric Brown and colleagues at McMaster University challenged the virtual screening community to predict the affinities of 50,000 molecules, none of which had been tested before but which were

TABLE 1.3 Hit Rates and Drug-Like Properties for Inhibitors Discovered with High-Throughput and Virtual Screening Against the Enzyme PTP-1B (Doman et al., 2002)

Technique	Number Compounds Tested	Hits with $IC_{50} < 100\,\mu M$	Hits with $IC_{50} < 10\,\mu M$	Rule of Five Compliant Hits[a]	Hit Rate[b]
HTS	400,000	85	6	23	0.021%
Docking	365	127	18	73	34.8%

[a]Number of 100 μM or better inhibitors that passed all four ''rule of five'' criteria (Paiva et al., 2001).
[b]The number of compounds experimentally tested divided by the number with IC_{50} values of 100 μM or less.

about to be tested in a high-throughput screen against dihydrofolate reductase (DHFR). In this experiment, the docking and HTS libraries were precisely the same, as were the experimental conditions. One of the startling results of this experiment was the very small number of hits to emerge from the screen. Indeed, whereas several groups were able to enrich putative inhibitors among their high-scoring molecules, the experimental group eventually concluded that they had no reliable hits at all (Elowe et al., 2005; Lang, et al., 2005). Intriguingly, several of the computational groups were able to indicate the lack of binders as part of their predictions (Brenk et al., 2005). Whereas the lack of experimental hits prevents definitive conclusions from this study, what does seem clear is that there is room for more of these comparative studies and ''competitions.''

1.6 CONCLUSIONS AND FUTURE DIRECTIONS

We have described the general steps involved in docking as well as typical algorithms for addressing each stage of the methodology. We have also listed examples in which prepackaged programs have been successfully used to discover novel inhibitors of a wide range of medically relevant applications. In the future, these methods will become progressively more integrated in the drug design process.

However, there are still several components of docking that need improvement. The two most-debated open questions in the field involve improving the scoring functions and developing algorithms for receptor flexibility. For scoring functions, research focuses on improving the treatment of solvent and the effects of entropy loss upon binding. Most docking approaches currently include drastic approximations of both of these properties, which have shown improvements over older methods. However, it is necessary to develop new schemes that treat these issues more accurately while preserving the speed of the calculation. Configurational entropy contributions are also difficult to calculate. Techniques that use molecular dynamics simulations to generate ensembles of ligand positions that are then rescored with high-accuracy scoring functions generate very accurate free energies of binding; however, they are computationally expensive (Kollman et al., 2000). It will be necessary to develop sampling techniques able to generate Boltzmann-weighted ensembles of ligand poses without requiring the use of expensive molecular dynamics calculations. Similarly, for receptor flexibility, many prepackaged programs have recently begun to develop new algorithms that allow for some measure of induced fit in the binding site. Several of these methods show great promise and will be further perfected with time, allowing for more and more structural rearrangement.

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