Virtual Screening Methods for Drug Discovery

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No one [in the pharmaceutical industry] would undertake the irksome task of making new products known to the medical profession without being, whether rightly or wrongly, convinced of their superior properties.

The Lancet, July 29, 1899, p. 318.

Abstract

Virtual Screening is a term applied to a diverse combination of computational methods to identify and rank potential drug candidates in a database, in part to reduce the magnitude and complexity of the screening problem, and to focus drug discovery and optimization efforts on the most promising leads. We review representative applications of compound database screening via molecular docking methods of increasing physical detail. In one application, a library of 350,000 compounds is screened for ligands targeting DNA gyrase, using rigid ligand docking according to a pharmacophore. In another application, a library of 153,000 compounds is screened for ligands targeting viral TAR RNA, first using rigid ligand docking to a simplified negative image of the target site followed by flexible ligand docking considering the target at atomic resolution. Finally, an application to screen a collection of 1.1 million compounds for agonists and antagonists of the estrogen receptor is described, using flexible ligand docking and atomic resolution descriptions of the target at every stage. The applications described indicate that virtual screening strategies are capable of recovering known ligands for both proteins and nucleic acids as well as discovering novel leads directly, or at least reducing the number of compounds for subsequent biochemical assay, commonly by one to three orders of magnitude.

Introduction

A key motivation for the study of protein structure and function is the exploitation of such information to design targeted drugs (Greer et al., 1994; Bohacek et al., 1996Shoichet and Bussiere, 2000). Traditionally, the work of drug discovery proceeds from a combination of both random screening and rational design. On one hand, the recent sequencing of the human genome has revealed that there are roughly 30,000 proteins represented there (IHGMC, 2000; Celera GST 2000), and their characterization is sure to have a profound impact on drug development (Beeley et al., 2000). On the other hand, a pharmaceutical company's compound library may contain upwards of one million entries, and combinatorial methods make it possible to access many orders of magnitude more compounds (Lebl, 1999; Jung, 1999). Clearly, exhaustive screening of all proteins with all reasonably accessible molecules is an immense task. The term *virtual screening* (VS) refers to a broad collection of computational methods to identify and rank potential drug candidates in a database, in part to reduce the magnitude and complexity of the screening problem, and to focus drug discovery and optimization efforts on the most promising leads (Walters et al., 1998). In particular, these efforts increasingly focus on the use of computational methods to predict target-based receptor-ligand interactions from three-dimensional models. We review here three approaches to this problem: fragment assembly within an active site, rigid body docking of ligands, and docking methods which directly incorporate flexibility of the ligand or the dynamics of the target.

The story of the development and use of aspirin is a good example of an early paradigm in drug discovery and refinement which is an interesting contrast to HTS and target-based approaches. It is a story which has taken on an aspect of legend (Rainsford, 1984). As early as 500 AD, the Greek physician Hippocrates records that preparations made from willow bark could be used to treat pain and fever. By the 1800s, European chemists had identified the compound salicilin, which is found in willow bark, and prepared its derivative sodium salicylate. By the 1870s, these chemicals were in mass production for use as analgesics, and they were especially valued as antipyretics, since the prevailing medical opinion suggested that fever was the most harmful aspect of the course of a given disease. In spite of its success at treating pain and fever, sodium salicylate was noted to cause stomach distress, in addition to having the unwelcome property of nauseating taste. Motivated to find a less-acidic, more well-tolerated alternative, in 1899 German chemist Felix Hoffmann of Bayer & Co prepared the derivative acetylsalicylic acid, which was quickly shown to have the antipyretic and analgesic properties of sodium salicylate, with less severe side-effects.

Although acetylsalicylic acid had been prepared previously by others, Bayer & Co were the first to market the compound as a medical alternative to sodium salicylate, and in that same year introduced it under the trade name aspirin, to great financial success. With the increasing acceptance of germ theory, and with the recognition that aspirin's use as an antipyretic did not generally shorten the course of a disease, use of aspirin shifted primarily to pain relief and reduction of inflammation, but as such continued to be widely used. However it wasn't until the 1970s that John Vane and others had described a

mechanism for aspirin's affects, by way of inhibition of prostaglandin, which in turn affects blood platelet activity and blood vessel elasticity (Vane and Botting, 1992). This understanding of aspirin's function ultimately led to additional uses in the prevention and management of stroke and heart disease. A rough timeline for aspirin's history is:

2300 years	Plant extracts containing salicins are used to treat pain and fever.
30 years	Salicylic acid is identified, and used to treat pain and fever.
1 year	Aspirin is prepared, medically tested, and introduced as a drug.
70 years	Aspirin's target and mechanism is described.
30 years	Additional uses of aspirin in stroke and heart disease are established.

In this case, aspirin's history culminated in the discovery of its target and mechanism. It is also interesting to note that less than a year of medical testing was needed at the time for aspirin's introduction as a drug in 1899.

In contrast, current approaches in drug discovery will commonly begin with knowledge of a target biological receptor, and research will proceed to first identify molecules showing activity with the target, followed by optimization of the best leads in terms of their binding affinity, and ultimately their efficacy and toxicological profiles. In cases where little specific information about the nature of the target is available, leads can be generated by random screening of compound libraries, and this strategy has become increasingly practical with advances in various High Throughput Screening (HTS) technologies for automated compound handling and assaying (Cox et al., 2000). Current automated systems can handle tens of thousands of compounds per day, so that it is practical to consider screening a substantial portion of an existing multi-million compound library in a reasonable amount of time. There are drawbacks to random screening however; compound libraries are costly and time-consuming to replenish, and

some targets require random screening of hundreds of thousands of compounds before reasonable hits are found; furthermore, libraries composed of natural products, traditional synthetics, and combinatorial products will each have their own limitations in terms of compound diversity (Lahana, 1999).

In cases where detailed structural information about the target is known, generally via X-ray crystallography or NMR, computational approaches can be used to complement HTS techniques, and this area of *structure-based drug design* has had a substantial influence on drug discovery and optimization in recent years. In particular, computational methods have been used to simulate and characterize a given compound's interaction with a target receptor, either approximately or in detail. Current day computer performance now makes it practical to apply these computational methods to large numbers of potential compounds, as a direct virtual counterpart to HTS; this computational-based screening approach is sometimes called *in silico* screening.

In this paper, we review some representative computational methods for virtual screening, and describe how these approaches have been used and evaluated. The computational methods can be divided into three classes, with increasing complexity and computational requirements; in practice they are often used in combination:

1. Evaluation based on two-dimensional (2D) property profiles (i.e. properties which can be calculated or inferred from the *molecular graph*, the traditional two-dimensional sketch of the molecule and its covalent connectivity) (Hall and Kier, 1991; Clark and Pickett, 2000).

2. Evaluation based on a target-specific pharmacophore, which is a reduced representation of the key features in the target system or ligand (van Drie et al., 1989; Sprague, 1995).
3. Evaluation based on detailed three-dimensional (3D) structure modeling of receptor-ligand interaction.

2D Property Profiles

Many of the 2D properties are employed in terms of general guidelines for whether a compound is "drug like", for example that it is synthetically accessible and orally bioavailable. A common example is the "rule of five"; compounds with two or more of the following characteristics are flagged as likely to have poor oral absorption (Lipinski et al., 1997):

- More than **5** H-bond donors.
- Molecular weight >**5**00.
- Lipophilicity: C log P (the calculated log of the octanol-water partitioning) > **5**.
- Sum of N's and O's (a rough measure of H-bond acceptors) > 10.

Extensions to these rules are used in REOS filtering (Rapid Elimination Of Swill), which also includes consideration of (Walters et al., 1998):

- Reactive or toxic moieties.
- Excessive numbers of rotatable bonds (floppy molecules may not bind strongly to a target due to entropic considerations).
- Similarity to existing drugs; interestingly, roughly half of all current drugs are encoded in the 32 scaffolds shown in Figure 1.

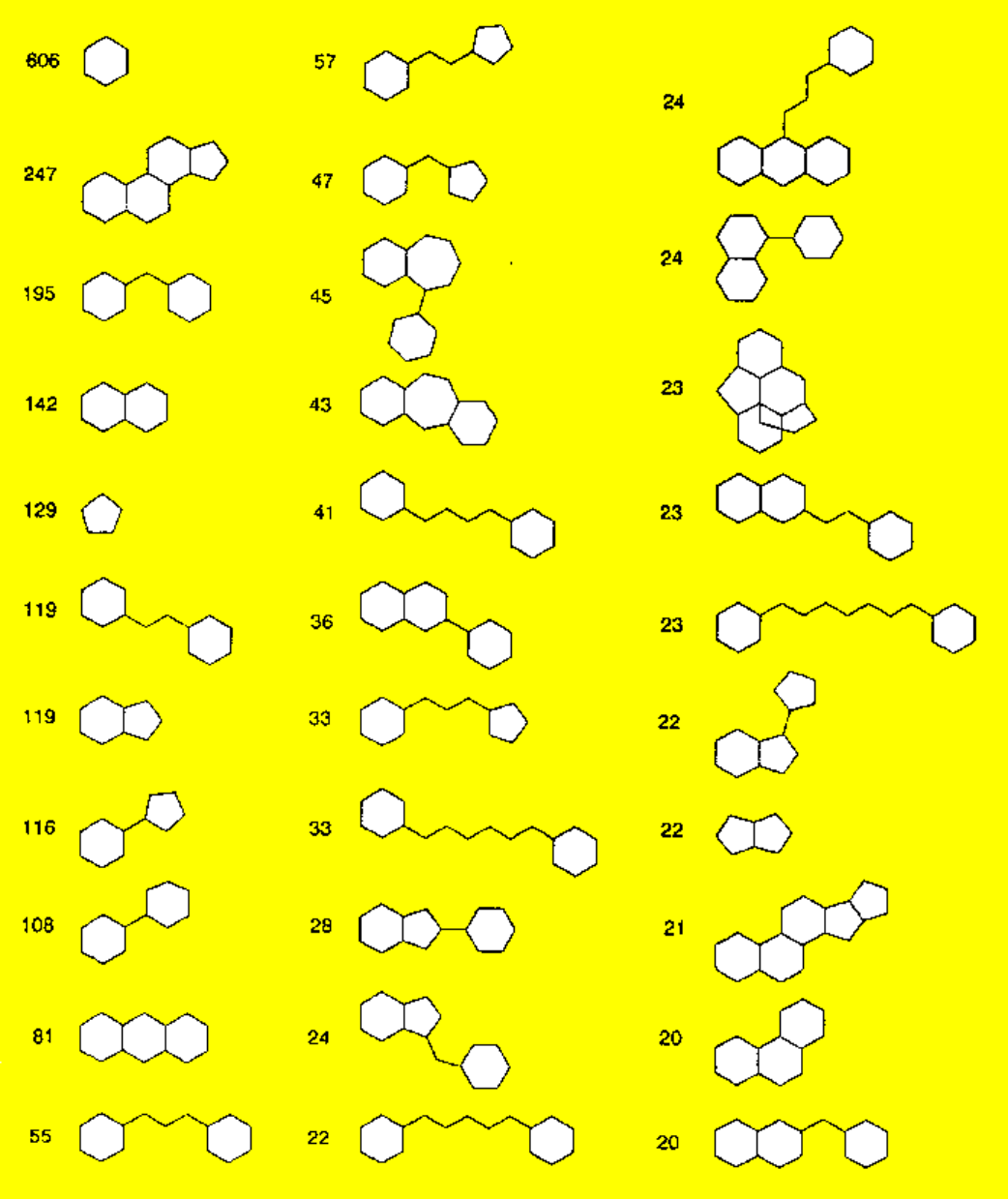


Figure 1. 32 scaffolds commonly found in drugs. The number beside each structure shows the number of drugs derived from it in a total of 2548 drugs included (Adapted from Walters et al., 1998).

An advantage of these 2D screening methods is that they can be applied rapidly and also in the absence of detailed information about the target. They can also be applied as a preprocessing step in both HTS and other VS approaches to reduce or focus subsequent screening steps by an order of magnitude or more.

Approaches using Pharmacophores

When information about the target is available, key structural features of the molecular systems can be encoded in simplified 3D descriptions called pharmacophores. These are commonly specified in terms of relative positions of just a few key structural elements such as H-bond donors and acceptors, ionic groups, and lipophilic centers. Such a simplified description allows rapid searching of a 3D compound database to identify molecules with complementary structural elements to the target pharmacophore.

Pharmacophores are commonly derived manually by inspection of the features common to a set of known ligands. As such, when used for screening, they are more likely to reveal compounds similar to known ones rather than novel leads. Furthermore, it may not always be possible to anticipate which particular structural features will best encode the nature of the target. Also, it is not easily possible to rank hits produced from this kind of search. Finally, it is common that a pharmacophore composed of three sites yields too many hits, while one with four sites yields too few, so that it is difficult to adjust the sensitivity of selection.

Approaches Based on 3D Docking

The most detailed and time consuming, but perhaps most the relevant VS methods involve objective docking of 3D ligand models with 3D receptor targets. Then, the quality of the fit between a given ligand and target can be used to rank that ligand, or in some cases to predict the actual binding affinity by calibration with known ligand-receptor complexes. The 3D docking approach to screening requires both a 3D structural

model of the target, as well as a 3D library of compound structures. As discussed below, in some implementations, rigid representations of the ligand and target are used for a given docking computation, and the dynamics and flexibility of the ligand and target are accounted for by performing separate docking computations on collections of conformations. In other cases, the conformations of the ligand can be varied as part of the docking computation. In another approach, a ligand can be constructed from smaller rigid fragments which are individually positioned into the target site.

Generally speaking, the primary limitation of 3D docking methods is computational speed. Therefore, an aspect common to many 3D methods is the use of simplified representations of the molecular systems for initial calculations, followed by a hierarchical application of increasingly more accurate but computationally time consuming models to increasingly smaller subsets of the library being screening. So, even with current computational technology, a key aspect of 3D docking as a screening method is a simple trade-off between computational accuracy and computational speed.

LUDI: Positioning and Linking of 3D Fragments

An early approach for positioning molecules into sites of a target is used by the program LUDI, which can be considered as a hybrid of pharmacophore and more complete 3D modeling methods (Boehm 1991; Boehm 1994). In the earliest version of the LUDI approach, the target site is characterized according to a collection of possible interaction sites for hydrogen bond formation or hydrophobic interaction. A given atom may have a number of potential interaction sites assigned; for example, in the case of an aliphatic

carbon, 14 equidistant interaction sites are defined in a spherical shell around the carbon atom, and any sites which fall inside the van der Waals limits of other atoms in the target are rejected. In the case of atom pairs forming hydrogen bond donors or acceptors, interaction sites are positioned according to ideal hydrogen bond geometries. As an alternative, interaction sites can also be defined on the basis of statistical distributions of hydrogen bond geometry and hydrophobic packing contacts from databases, initially such as the Cambridge Structural Database (CSD).

A given molecule to be positioned in the target site is also characterized in this way, and a simple distance-based test is applied to see if there are arrangements of two, three, or four interaction sites in the molecule with pairwise distances that correspond to distances between sites in the target. If this test is passed, then as a next step rigid body rotation and translation is used to position the molecule into the target to optimize the root mean square (RMS) distance alignment between the sites in the target and complementary sites in the given molecule. A fit is accepted if the RMS value is below a specified threshold, and if there is no substantial van der Waals overlap between the molecule and the target. In initial implementations, in order to reduce the computational time of alignment, the computation focuses on a subset of less than 10 atoms in the molecule being aligned. Since it is not easily possible to anticipate in all cases which atoms are relevant, the computation may be repeated with a different subset of atoms for the same molecule. It should be noted however that the compromise of atom subset selection is not a requirement of the LUDI method, but rather just an early approach to reduce computational time (since LUDI was introduced in 1991, laboratory computers have

increased in speed by roughly two orders of magnitude). In original implementations, this method is used to select and position fragments from a library of several hundred small molecules (ca. 5-30 atoms) and functional groups with ideal geometry, some of which are stored in multiple conformations. After two or more fragments are positioned in this way, nearest contacts between adjacent fragments are connected by bridging fragments, also using ideal geometry. In this way, larger more complicated ligands can be identified using only a small library and without the need to consider overall ligand flexibility directly. An example of the LUDI interaction site selection and alignment method is shown in Figure 2, which shows the results of a test attempting to recover the orientation of a molecule of benzoic acid within its known crystal packing matrix. Figure 2A shows the crystal packing matrix with a cavity generated by removing one molecule. Figure 2B shows the interaction sites generated for the cavity, which includes 14 aromatic interaction sites, one hydrogen bond acceptor (A-Y, blue) and one hydrogen bond donor (D-X, red). Figure 2C shows the final result of alignment to the specified interaction sites. The molecule of benzoic acid positioned in this way by LUDI agrees with the actual position of the molecule in the crystal to an RMS of 0.23Å, as shown in Figure 2D. The LUDI approach provides important advantages. One is computational efficiency. Another is the ability to construct potentially novel ligands from small libraries of fragments, in a virtual counterpart to combinatorial chemistry. In its basic form however, the fragment assembly method can result in physically unreasonable or strained molecules or conformations. Furthermore, the fragment assembly does not anticipate entropic requirements of binding.

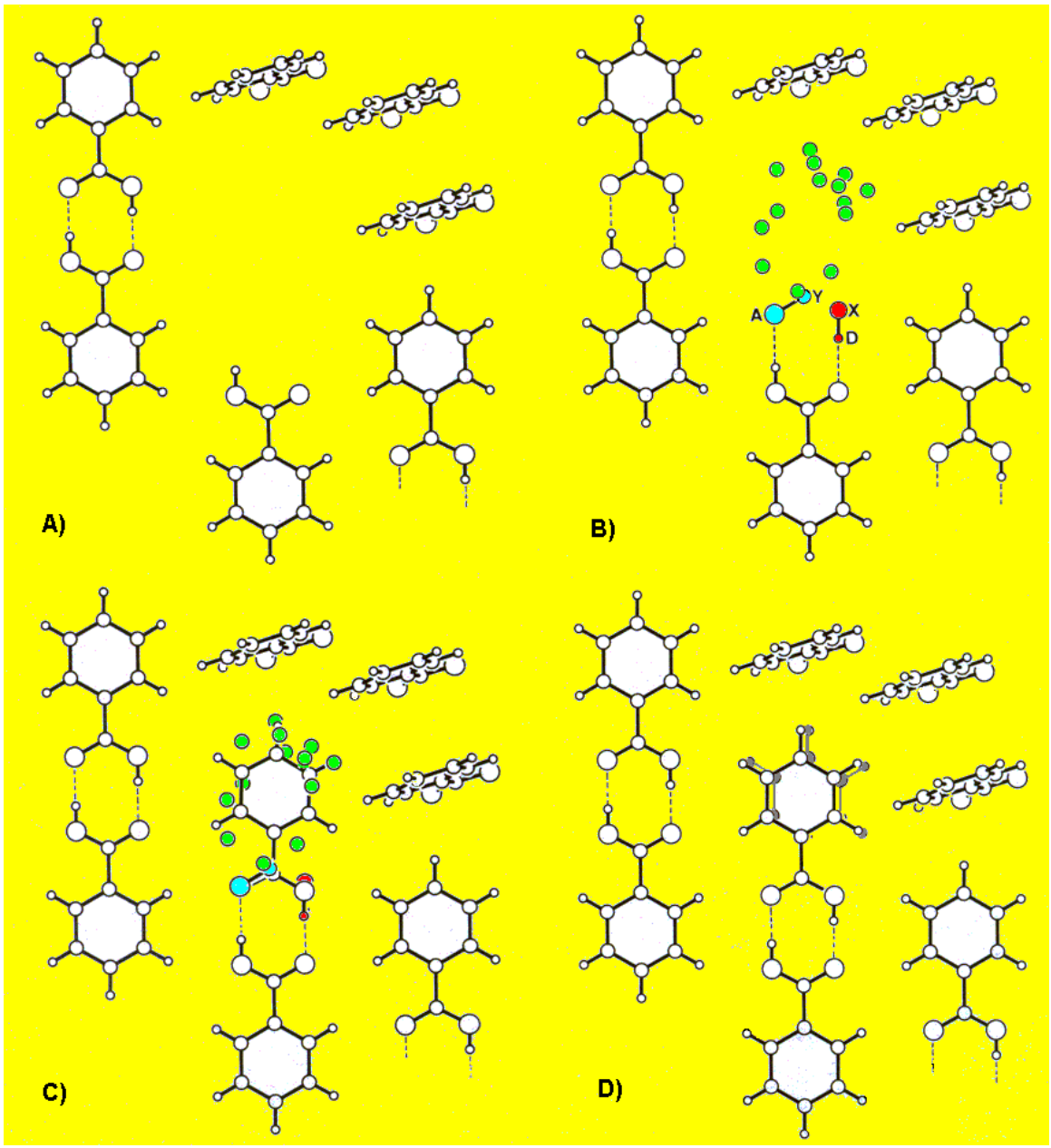


Figure 2. Example docking of benzoic acid into a crystal packing cavity. A) The crystal packing matrix, with one molecule removed. B) The identified aromatic interaction sites (green), hydrogen bond acceptor site A-Y (blue) and hydrogen bond donor site D-Y (red). C) The molecule as positioned by LUDI. D) A comparison of the computed position and the known X-ray position of benzoic acid in the matrix (gray). Adapted from Boehm, 1991.

In one application of LUDI as part of a drug discovery process, *in silico* screening was used to help uncover novel inhibitors of the bacterial enzyme DNA gyrase (Boehm et al., 2000). This enzyme is an attractive antibacterial target, as it is essential for prokaryotic

DNA replication, transcription, and recombination, and is not present in mammals (Reece and Maxwell, 1991; Maxwell, 1993). The active enzyme is an A_2B_2 complex, where the 97 kDa subunit A is involved in DNA breakage and reunion and the 90 kDa subunit B acts in ATP hydrolysis (Wigley et al., 1991; Lewis et al., 1996). There are existing and successful drugs, for example quinalones, which inhibit DNA binding to gyrase at subunit A, but bacterial resistance to these is emerging (Ho et al., 1999). There are also drugs, such as novobiocin, which inhibit ATP recognition at subunit B, but these suffer from toxicity as well as rapidly developing resistance. Random screening to uncover alternatives to the known inhibitors of DNA gyrase did not produce any hits. Therefore, an approach including an *in silico* screening directed at the ATP binding site of subunit B was employed as part of an overall scheme for ligand discovery based on identifying many low molecular weight inhibitors, with the anticipation that the ensemble of hits will suggest larger ands more potent inhibitors. This is in direct correspondence to the low molecular weight fragment screening and assembly used in the LUDI approach. These low molecular weight compounds are termed *needles*, because they are likened to sharp needles which can penetrate into deep and narrow channels, subpockets, and grooves in the target site. So, the authors term this approach *needle based screening*. It is expected that these needle compounds will generally bind with low affinity, so the *in silico* screening is accompanied by biochemical assay methods which can detect weak binding.

The starting point for the *in silico* screening includes 3D X-ray structures of subunit B with the ATP substrate analog ADPNP, as well as two structurally different inhibitors cyclothialidine and novobiocin. From inspection of these complexes, a pharmacophore

including two key hydrogen bonds and one lipophilic contact was proposed. LUDI was used to search a 350,000 compound database composed entries from the Available Chemicals Database (ACD) and the Roche Compound Inventory (RCI), and kinase inhibitors known from the literature. The search resulted in a list of about 200 molecules consistent with the pharmacophore. The search was repeated using the program CATALYST (Sprague, 1995), which uses an extended description of possible interaction sites, resulting in an additional list of 400 molecules. The search procedures involved roughly one week of computation time on 10 SGI R5000/180 processors.

After the search, related analogues to the 600 best hits were also assembled, resulting in a total of 3000 compounds which were then analyzed by a spectrophotometric ATPase assay sensitive to weak binding (Nakada et al., 1995). This assay identified 150 weakly binding compounds. Both X-ray crystalography and 2D $^{1}H/^{15}N$ NMR was used to confirm that binding of a given compound occurred at the ATP binding site. NMR was also used to estimate binding affinities by chemical shift perturbation observed in a titration series, and this information was used to rank the compounds (Zhou et al., 1996).

The next stage was an optimization of the best molecules guided by detailed consideration of the 3D structure of the various complexes. The optimization first aimed to support observed networks of hydrogen bonds and side-chain stacking onto a salt bridge in the binding site. A second optimization involved conserving a hydrophobic interaction which was observed in one derivative that resulted in better than expected inhibition.

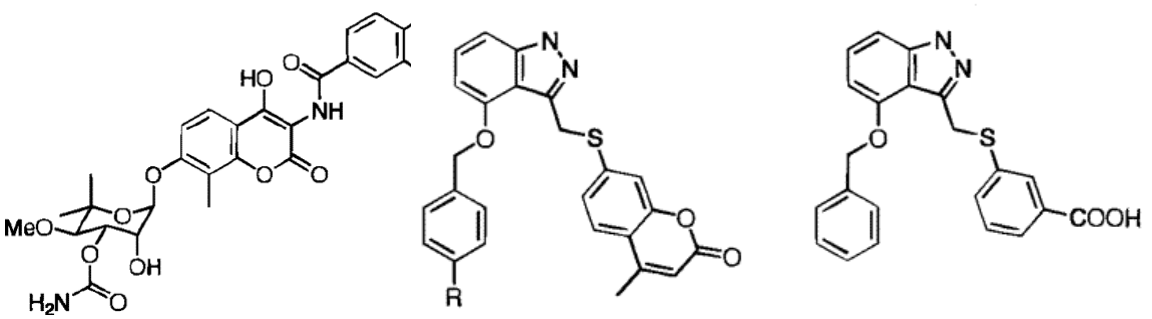


Figure 3. Left: novobiocin, a DNA gyrase inhibitor. Center: a family of compounds identified by *in silico* screening and synthetic optimization; for the compound R=tBut, biochemical assay indicated that it is a 10 times more potent inhibitor than novobiocin. Right: this related compound was cocrystalized with the DNA gyrase target; a structure of the binding site region of the complex is shown in Figure 4.

The final result of the screening and optimization process is a series of compounds that includes one which is a 10 times more potent inhibitor of DNA gyrase than novobiocin, while at the same time, structurally less complex; the compounds are shown in Figure 3. Furthermore, X-ray crystallography of one of these compounds confirms the desired hydrogen bonding network, ligand/salt bridge stacking, and hydrophobic interactions; an illustration of the binding site of the complex structure is shown in Figure 4. One thing to note about this successful application of *in silico* screening as part of a drug discovery initiative is that more than one database search approach is employed. In this case, LUDI was used to perform a pharmacophore-based search, and the program CATALYST was used to augment these results based on a more complete list of hydrogen bond donor and acceptor sites in the target, etc. As described above, software systems such as LUDI have features which anticipate automation, such as automated identification of interaction sites or automatic analysis and linking of fragments. It should be noted however that in practice, the steps of defining a pharmacophore or optimizing a compound by linking or substitution are still closely informed and guided by the knowledge and experience of the chemist.

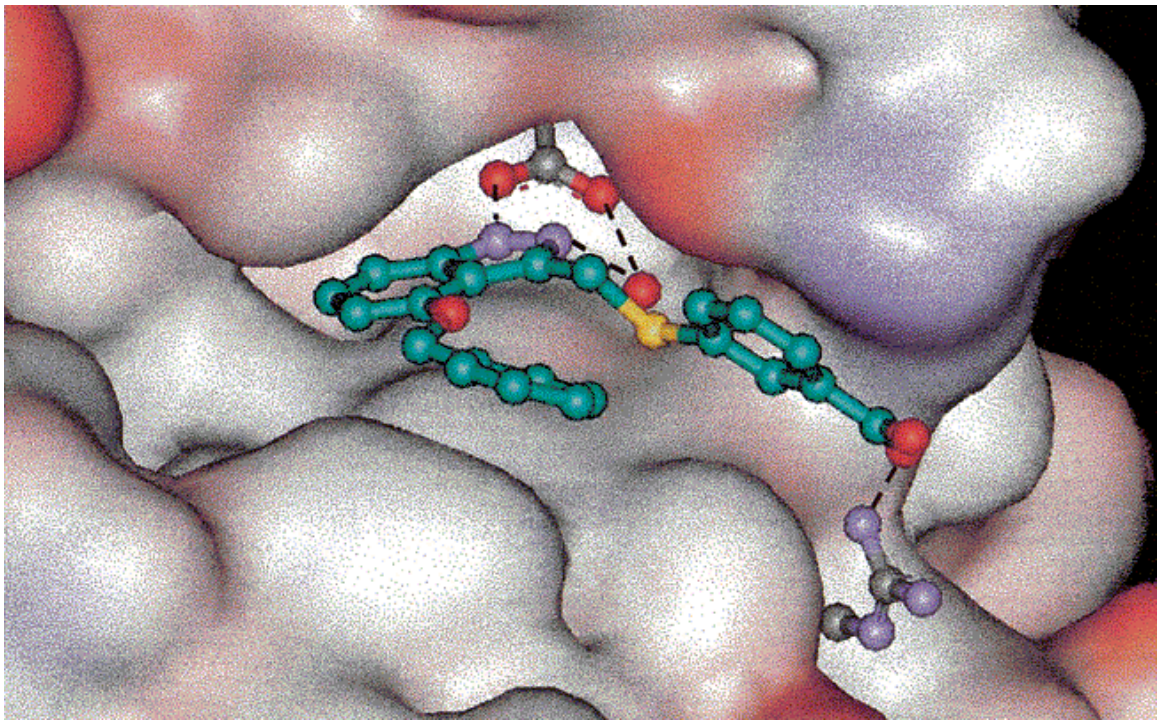


Figure 4. Detail of the X-ray structure of the complex between the DNA gyrase target and one of the compounds in Figure 3 found by *in silico* screening and synthetic optimization. This structure confirms the original binding hypothesis regarding the hydrogen bond network and ligand stacking with the target salt bridge. From Boehm et al., 2000.

DOCK: Rigid Body Docking to a Negative Image of the Target Site

The program DOCK uses a description of the target site cavity for docking purposes (Meng et al., 1992; Shoichet et al., 1992). This description forms a "negative image" describing the empty space in a target site into which the proposed ligand must fit. This image of a target site is prepared using a utility program which creates clusters of overlapping sphere-shaped spaces which are in contact with the target's surface. Clusters of these spheres populating a target site are then selected interactively, and collectively, the space they define is used as a description of the target cavity. An example of this characterization for HIV protease is shown in Figure 5.

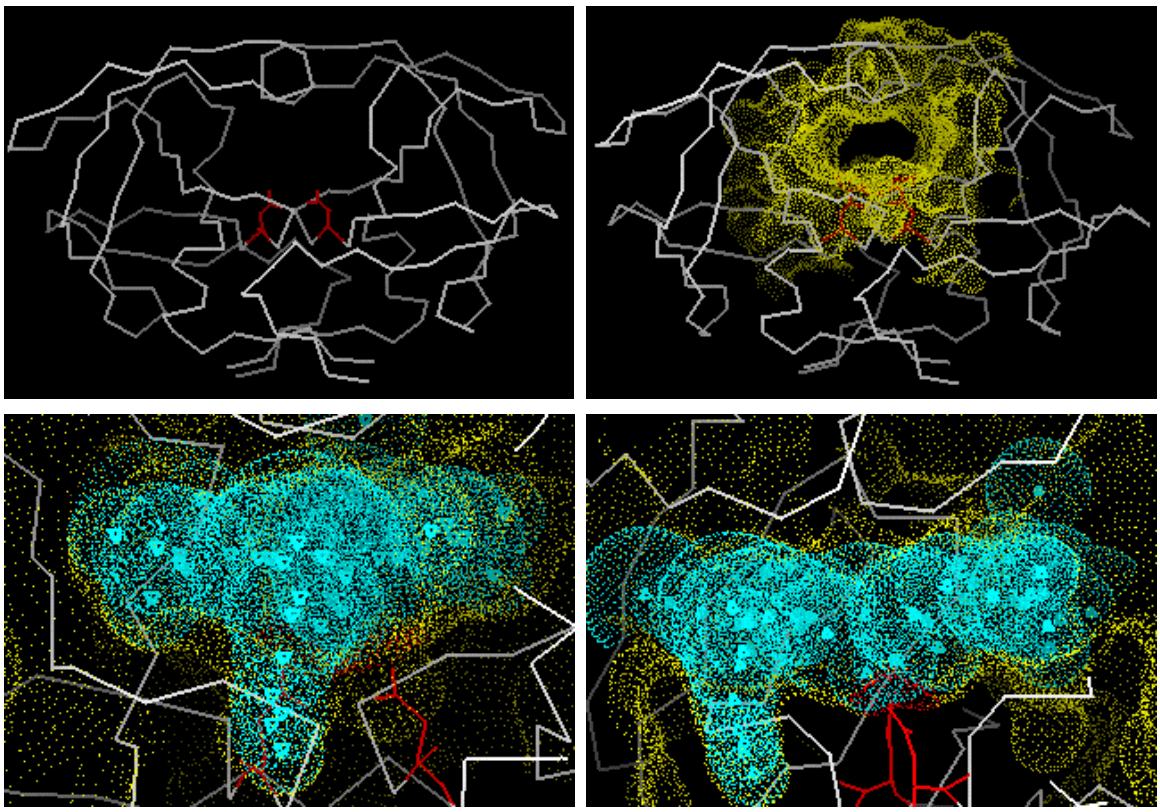


Figure 5. Example construction of a negative image of a site in HIV protease. Top left: the protein backbone structure. Top right: analysis to identify a series of points (yellow) defining the protein surface. Bottom: the surface is populated by spheres in contact with the surface (blue) to define the shape and extent of the target cavity; two detailed views are shown.

Given this characterization of the target, the DOCK program is then used to position a rigid 3D model of a given molecule into the space defined by the spherical collection. Once a molecule is positioned into the negative image in this way, the results can be scored according to one or more force field terms such as van der Waals and electrostatic interactions, and surface area contact between ligand and target. Because it uses a simplified description of the target cavity rather than the receptor structure itself in atomic detail, DOCK has the advantage of being fast, and the approach allows the description of the target site to be tuned by including additional spheres of smaller radius. Furthermore, the use of a multi-sphere representation of a target cavity along with force

field terms to evaluate results makes the method more objective than a pharmacophore-based approach.

One application of the DOCK program was as the first part of a hierarchical *in silico* screening for ligands targeting the RNA molecule TAR (Filikov et al., 2000). The TAR molecule (RNA transactivation response element) forms a complex with the RNA binding protein tat (transactivating regulatory protein), in an interaction which is necessary for viral replication of HIV-1 (Kao et al., 1987; Marciniak and Sharp, 1991; Carroll et al., 2000). One interesting aspect of this application is that it targets a nucleic acid rather than a protein. Starting with 153,000 compounds from the ACD, screening via DOCK was used to dock, evaluate and select a subset of 30,000 compounds for subsequent rounds of more detailed *in silico* screening, as described in the next section.

ICM: an Example of Flexible Ligand Docking

The ICM program performs docking by a combination of Monte Carlo manipulation of the ligand torsion angles to account for ligand flexibility, plus alternating rounds of local minimization to optimize an energy function (Totrov and Abagyan, 1994; Totrov and Abagyan, 1997). The energy function can include van der Waals terms, electrostatics, entropic terms based on numbers of rotatable bonds, solvation terms based on solvent accessible surface area, etc. As such, it can provide a more physically realistic evaluation of a given proposed complex than rigid body docking, even when the same energy terms are used to rank a result. However the effectiveness of conformational sampling for the flexible ligand depends on the number of optimization steps which can be performed.

To validate this flexible ligand docking approach in preparation for a TAR screening scheme, the method was first tested on five known RNA/small molecule complexes from the Brookhaven Databank (PDB). In the tests, both the starting positions and the conformations of the ligands were randomized before the docking computation. In four out of the five compounds tested, the ligand conformation and position derived via docking was qualitatively similar to the known structure of the corresponding complex. These same five compounds were also used to calibrate the docking calculation of binding energy, as described below.

In the complete *in silico* TAR screening scheme, the 30,000 hits determined by DOCK were evaluated by ICM to select a narrower subset of 5,000, and these were reevaluated by ICM using 10 times the number of dynamics steps, to select the best 350 ligands. This subset included all known ligands for TAR RNA. Eight compounds were selected for biochemical assay from the best 350 results of in silico screening; the compounds were selected simply on the basis that they were readily available, and known to be soluble and non-toxic. Two of the compounds tested yielded CD_{50} values (concentration of ligand which reduces tat/TAR binding by 50%) in the μM range, which would be considered a suitable lead for drug design.

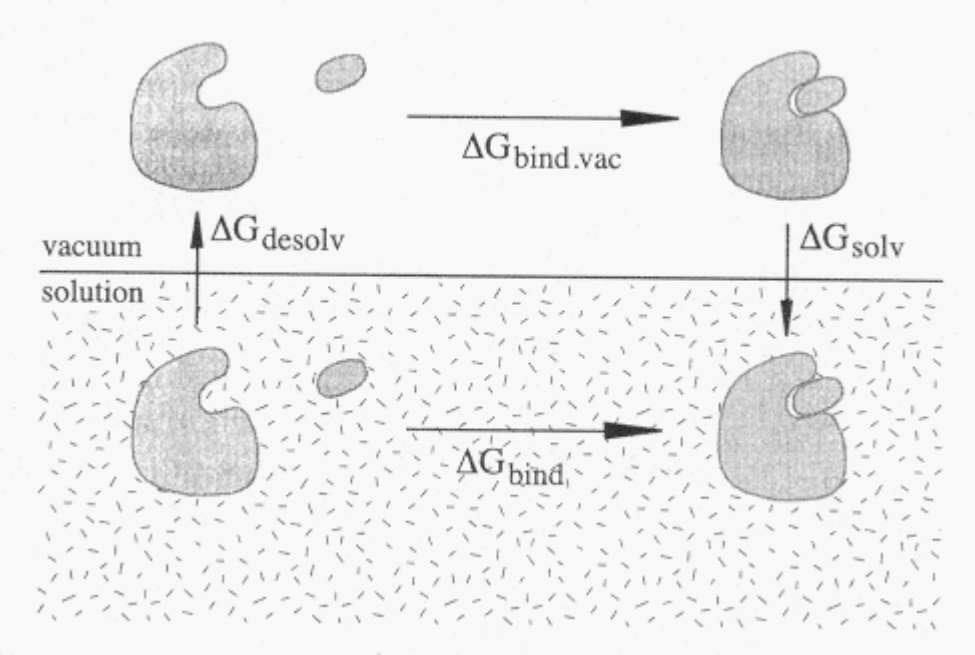


Figure 6. Thermodynamic cycle for computation of binding energies. The cycle equates the energy of complex formation in solution with the steps of desolvating the target and ligand individually to vacuum, binding in vacuum, and resolvation of the complex. From Filikov et al., 2000.

Computing the Free Energy of Binding

As part of the tat/TAR in silico screening, an empirically calibrated computation for the free energy of binding was established, in an attempt to make the ligand ranking more physically realistic, and therefore more predictive of actual binding activity. This was done based on the thermodynamic cycle shown in Figure 6, so that the free energy of binding ΔG_{bind} can be expressed in terms of the energies of transferring the unbound system from the solvent to vacuum ($\Delta G_{desolv)}$, the energy of complex formation in vacuum ($\Delta G_{bind.vac}$), and the energy of transferring the bound system back to the solvent (ΔG_{solv}):

$$\Delta G_{bind} = \Delta G_{desolv} + \Delta G_{bind.vac} + \Delta G_{solv} \quad (1)$$

The desolvation and solvation terms are calculated according to atom solvation terms for 11 different atomic classes derived experimentally from small molecule studies. It is

noted that this does not account for entropic gains due to the displacement of ordered water molecules on the biomolecular surface, which can be considerable; this is therefore incorporated as an additional term ΔG_{cav} which is proportional to the difference in solvent exposed surface area between the free and bound states.

The free energy of complex formation in vacuum is decomposed into:

$$\Delta G_{bind.vac} = \Delta G_{el} + \Delta G_{vdw} + \Delta G_{hb} + \Delta G_{iso} + \Delta G_{rot} + \Delta G_{tor} \quad (2)$$

The terms ΔG_{el}, ΔG_{vdw}, and ΔG_{hb} describe intermolecular electrostatic and van der Waals interactions, and hydrogen bond energies. ΔG_{iso} is a term describing intramolecular strain. ΔG_{rot} is a term describing the energy associated with the loss of the overall rotational and translational degrees of the ligand on binding. Finally, ΔG_{tor} describes the loss of entropy for rotatable bonds which become fixed on binding. Based on this description of binding energy, values were found for the five computed RNA/ligand complexes, and these numerically estimated energies were then scaled to give a best match to the known measured binding constants of the five ligands. The resulting scaled energy term reproduces the measured binding energy to an RMS error of 2.7 kcal/mol.

The DockCrunch Project: Virtual Screening of a 1.1 Million Compound Database

The DockCrunch project is a demonstration of the feasibility of using in silico screening effectively on multi-million compound databases (Waszkowycz et al., 2001). The study used molecules drawn from the 1.5 million entries in the ACD Screening Compounds library (ACD-SD). The ACD-SD was first filtered by 2D profiles to remove inorganic compounds, compounds with molecular weight < 200 or > 600, with C log P > 7, with a

hydrogen bond acceptor count > 8, or with a hydrogen bond donor count > 8. This resulted in a collection of 1.1 million compounds for docking.

The estrogen receptor was chosen as the screening target; its behavior is well-studied, and X-ray structures of the receptor and its complexes are available (Brzozowski et al., 1997; MacGregor and Jordan, 1998; Grese and Dodge, 1998). The binding of an agonist (a ligand which activates the receptor on binding) induces a structural change in the receptor which allows other recognition proteins to transport the complex into the cell nucleus, where it binds to a specific sequence of DNA and modulates gene expression. Significantly, ligands which bind to the receptor without activating it, or showing only partial activity, induce a different structure in the receptor than does agonist binding. For purposes of this study, those ligands which bind with partial or no activity are all classified as antagonists. So, as an additional aspect of the DockCrunch project, screening was carried out twice using two different human alpha-estrogen receptor X-ray structures; one complexed with the agonist estradiol (PDB code 1ERE), and another complexed with the antagonist raloxifene (PDB code 1ERR). Then, 20 known agonist compounds and 20 known antagonist compounds were intentionally added to the database, to see how they would be identified during subsequent VS steps.

The docking and evaluation was performed with the program PRO_LEADS, which docks a flexible ligand into a rigid receptor (Baxter et al, 2000). Like ICM, PRO_LEADS uses a series of random torsion space adjustments to explore conformations of the ligand. To make this process more effective, during an optimization PRO_LEADS maintains a list

of conformations already evaluated (a *tabu* list) in order to promote a diverse exploration of structural space. The approach commonly requires 10^5 to 10^6 steps in a given docking sequence. In the study, a given molecule was docked five times in this way, each time starting from a different randomized conformation and position, and the best result out of the five trials was retained. The results were ranked via an energy scoring function which directly predicts the free energy of binding, using terms for hydrogen bond geometry, lipophilic contacts, and entropic terms based on the loss of ligand flexibility on binding. The docking and energy evaluation scheme was validated on a set of 82 complexes with measured X-ray structures and binding constants. For these cases, binding energies were predicted to an RMS error of 8.7 kJ/mol. Furthermore, roughly 80% of the ligands positioned by docking agreed with the known positions measured by X-ray to an RMS of 2 Å or better.

The PRO_LEADS docking computations for the 1.1 million compound library required roughly one week of time using 64 SGI R10000 processors. In scoring schemes based on calculated overall binding energy alone, the best 1% of the ranked database contains 15 of the 20 known reference ligands. All 20 of the reference ligands are included within the best 20% of the ranked database. If the database is re-ranked by considering individual components of the calculated energy (hydrogen bonding, steric clash, and polar/lipid mismatch terms), all 20 of the reference ligands are again recovered in the best 1% of compounds.

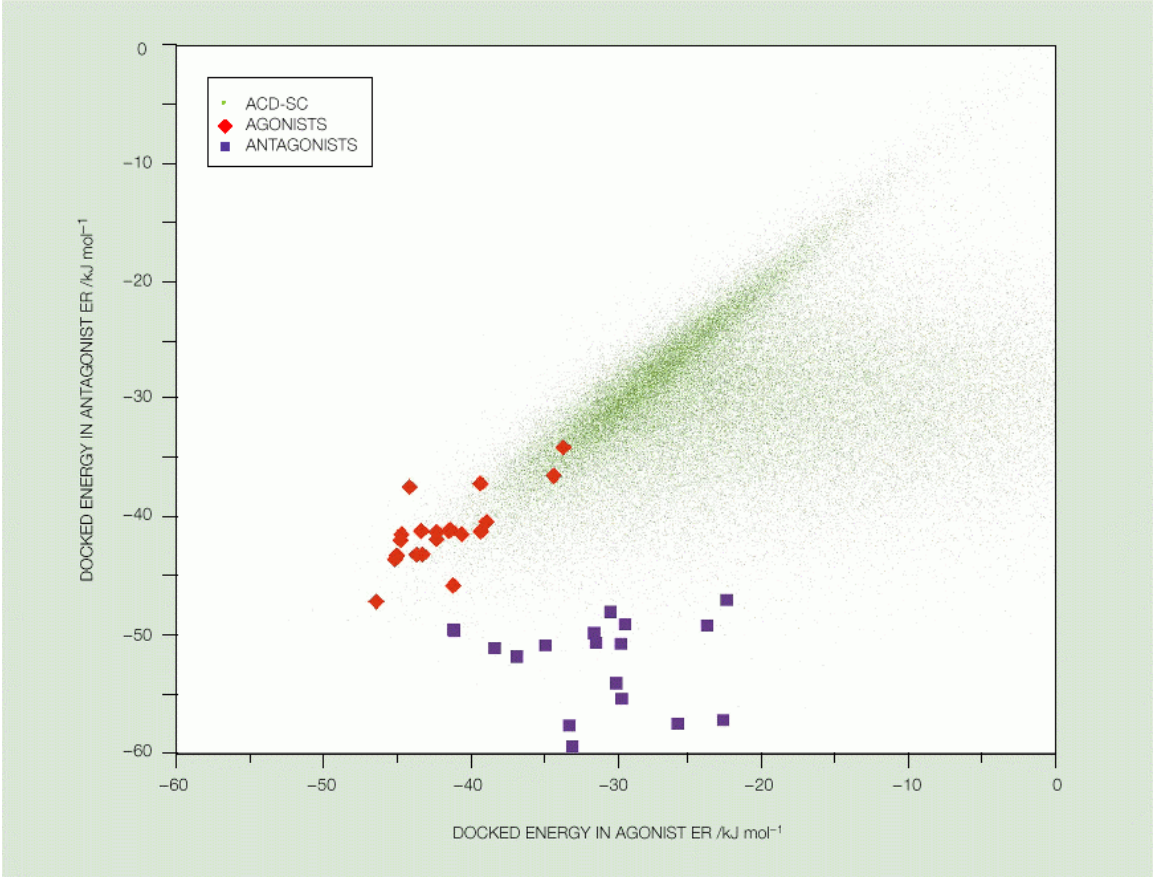


Figure 7. Energy of compounds docked to both antagonist and agonist forms of the estrogen receptor. As shown, the known antagonist compounds (blue squares) have more favorable binding energies for the antagonist form of the receptor, however binding energies for known agonists (red diamonds) are similar for both forms of the receptor. Random compounds from the ACD-SD (green dots) also show this trend. From Waszkowycz et al., 2001.

An interesting aspect of this in silico screening is a comparison of the results for the agonist and antagonist forms of the estrogen receptor, as shown in Figure 7. Figure 7 shows a scatter plot of a given ligand's computed binding energy to the agonist receptor versus that same ligand's binding energy in the antagonist receptor. As shown, reference antagonist ligands have favorable antagonist binding energies, but do not dock as well to the agonist receptor. However, the computed binding energy for the reference agonist ligands is about equally good for both forms of the receptor. This trend can be seen over the collection of compounds screened. The difference in specificity can be understood by noting that the agonist compounds are generally smaller than the antagonists, and

correspondingly, the target site of the antagonist is larger than that for the agonist, and so it can accommodate a wider variety of ligands.

As further validation of the screening results, a group of high scoring compounds was selected on the basis of individual energy terms, 2D property profiles, similarity of binding modes to the known ligands, and structural dissimilarity to the known ligands. This resulted in a collection of 300 compounds for the agonist receptor, and 120 compounds for the antagonist receptor. Finally, based simply on compound availability, 21 agonist compound and 16 antagonist compounds were selected for biochemical assay. Of the 37 compounds assayed, 21 showed an inhibition constant of 300 nM or less, with the best binding affinity being 8 nM. This clearly indicates that the virtual screening strategy employed can successfully identify potent ligands, and it also compares favorably with the VS schemes described above which require varying degrees of guidance and interaction based on chemical insight.

Concluding Remarks

We have reviewed three applications of virtual screening and docking, in order of increasing physical detail, and correspondingly increased computational requirements. Some important themes from these applications are:

- VS methods can successfully reduce the complexity of subsequent biochemical assay by effectively selecting subsets of a compound library, commonly reducing the number of compounds by a factor of 10 to 1000.

- VS docking methods can reproduce the binding modes of known ligands, and are sufficiently fast to allow screening of a million compounds or more.
- VS docking methods can compute binding energies to a useful degree of accuracy, and these energies can be used to rank compounds effectively.
- VS approaches are capable of recovering known ligands and discovering novel ones, sometimes with binding in the μM to nM range.

It can also be noted that in addition to screening for binding to a therapeutic target, it is anticipated that these same techniques can be used to screen for interactions with proteins involved in drug metabolism, to anticipate and optimize the ADME/Tox profiles (absorption, distribution, metabolism, excretion, and toxicity).

It must also be noted that in all cases, the screening methods still have many subjective adjustable parameters, which can vary their effectiveness substantially. The applications presented here are indications of the best results possible so far with current technology.

There are a number of factors which will likely serve to increase the effectiveness, attractiveness, and importance of VS methods. One key factor is the complementary improvement in automated methods for biochemical screening. Another simple but critical factor is available computational speed: inexpensive PC hardware already provides superior computing performance relative to the laboratory workstations cited in the examples above. Once suitable software and compound libraries are prepared, and VS protocols are established, an *in silico* screening on such PC hardware is of negligible cost, but potentially great value in subsequent physical screening. Finally, as genomic

analysis continues to identify medically relevant targets, there will be increasing need for effective screening methods, suggesting an expanding role for virtual screening in the future.

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