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Abstract

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Title:
Predicting how drug molecules bind to their protein targets

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Abbreviations
ADME – absorption, distribution, metabolism, excretion
GBSA – Generalised Born solvent accessibility surface area
MD – molecular dynamics
MCSS – multiple copy simulation search
PDB – Protein Data Bank (http://www.rcsb.org)
PBSA – Poisson-Boltsman solvent accessibility surface area
SBDD – structure-based drug design
VS – virtual screening

Abstract (100-120 words)

There have been substantial advances in the application of molecular modelling and simulation to drug discovery in recent years, as massive increases in computer power are coupled with continued development in the underlying methods and understanding of how to apply them. Here, we survey recent advances in one particular area – predicting how a known ligand binds to a particular protein. We focus on the four contributing classes of calculation: predicting where a binding site is on a protein; characterizing where chemical functional groups will bind to that site; molecular docking to generate a binding mode for a ligand and dynamics simulations to refine that pose and allow for protein conformation change. Examples of successful application are provided for each class.

Highlights

1. Mixed solvent dynamics can characterise what will bind to a site on a protein
2. Dynamic docking approaches can allow for protein flexibility in ligand binding
3. Modern computing allows more realistic estimates of protein-ligand interaction energy

Introduction

The majority of drug discovery projects begin with identification of a small molecule compound which binds to a defined site on a specific biological molecule (usually a protein), affecting the function of that target protein. This initial hit is then optimized to incorporate adequate drug-like properties (affinity, selectivity, efficacy, ADME, etc.) into a candidate compound that generates the desired therapeutic effect and is suitable for clinical trials.

Over the past thirty years, there has been a steady increase in the use of structure-based methods in this drug discovery process where models of how compounds bind to the target can allow rational design of the required improvements in the compounds. For some targets, experimental methods can provide structural information with sufficient throughput and speed to interactively guide the structure-based design. For example, X-ray crystallography provides an atomic level picture of how compounds bind and NMR spectroscopy can provide varying levels of information on interactions between the compound and the protein, such as whether a compound binds, where it is binding to and (in some limited cases) a structure of the compound binding to the target. However, it is often not possible to generate such structures with sufficient speed to inform decisions about compound optimization.

In this review, we survey recent developments in the computational methods that predict how compounds bind to their protein target using either an experimentally determined structure of the target or a model based on sequence homology. Some of the methods can be used to screen compound libraries (real or virtual) for initial hits; in addition, the methods can help to guide optimization of compounds in structure-based design. These applications are not discussed in detail here. What we focus on are the methods that, once a compound is demonstrated to bind, can be used to predict the position and orientation or "pose" of the compound binding. As summarized in Figure 1, we have loosely divided these methods into four categories: (1) identifying binding sites; (2) characterizing the potential of a binding site to bind chemical matter; (3) predicting the position and orientation (or pose) of compound binding and (4) dynamic docking to explore both the energetics of binding and conformational change to refine the pose. Before summarizing these in turn, we first survey some history and the issue that underpins all molecular modelling – the ability to estimate energy of interaction.

Origins of the methods

A more detailed description of the origins of structure-based design methods is provided elsewhere[1] but there are three influential developments that should be highlighted – CHARMM[2], GRID[3] and DOCK[4]. The Karplus group developed molecular dynamics (MD) simulations of a protein in 1977[2], which led to the development of the CHARMM[5] (Chemistry at Harvard Molecular Mechanics) program which became a central platform for many molecular simulation methods over the following decades. One of the most influential developments for structure-based drug discovery in the 1980s was the program GRID from Goodford[3]. This introduced the idea of characterizing what types of chemical functionality would bind to a binding site by calculating the energy of interaction between the protein and a functional group at each point on a grid. Finally, there is the DOCK program from the

Kuntz group[4] which was the first widely used program for computationally docking compounds into the structure of a protein. Although some of the ideas within these programs were built on the work of others, the programs (and their authors) became major promoters of the ideas of using computational methods to characterize and predict how compounds can bind to proteins and formed the foundation of the current generation of methods.

Predicting the energy of interaction between protein and ligand – scoring functions

All structure-based design methods critically rely on an estimate of the energy of interaction between a ligand (or probe) and the protein. Most approaches still rely on the rather simplistic treatment established in the early methods[2-4] where the non-covalent interactions are treated with simple Coulombic (for electrostatics) or Lennard-Jones (for van der Waals) interaction potentials but there is increasing use of more sophisticated treatments. The theoretical bases for these more advanced calculations were established a long time ago. What has changed in recent years is the relentless increase in computer power allowing these methods to be applied within a realistic timeframe. There are three main areas to highlight.

The first are the perturbation methods[6] which calculate changes in free energy by performing extensive MD while transforming (in this case the compound) from one chemical structure to another. The second is a number of approaches for more extensive treatment of electrostatics: for example with Poisson-Boltzman or Generalised Born models within a molecular mechanics calculation (called MM-PBSA and MM-GBSA) – this can account for bulk solvent effects[7]; or using explicit quantum mechanics for part of the model within a molecular mechanics model to account more fully for both electrostatics[8] and metal ion charges[9]. Finally, there has been a growing realization that water molecules, both their stability and their interaction networks, can make important contributions to the thermodynamics of ligand binding[10,11]. However, even with these advances, our ability to predict the thermodynamics from a static model of a protein-ligand structure is still far from predictive. Nonetheless, the methods have been central to recent successes in structure-based design, such as potent non-nucleoside reverse transcriptase inhibitors [12] and other examples described below.

Pocket Detection

The first challenge of SBDD is identifying where the ligand binds, for which geometry-based and energy-based programs have been successfully developed[13]. Geometry-based programs such as CAVITY[14] or fpocket[15] identify the largest and deepest cavities within a static structure; examples of successful use are identification of two new allosteric sites in the crystal structure of PHGDH in which virtual screening (VS) identified compounds with anti-tumor activity[16] and cavity detection in models of the intrinsically disordered protein Myc for which VS identified cell active compounds[17]. Another method uses hidden Markov models to identify cryptic pockets which can then be exploited as allosteric sites[18]. The more difficult to identify pockets are where a flexible model of the protein is needed to identify previously unidentified cavities. In these cases, energy-based methods similar to those used to identify protein-ligand hot-spots[19,20] can be used to map the protein surface repeatedly while exploring low energy conformers of side chains. Such calculations complement some of the experimental methods which explore transient sites and can be incorporated into strategies for

ligand design[21,22]. Finally, a comprehensive review[23] summarises the large number of methods developed to compare binding sites on the basis of protein structural information, with many examples of how this has been used in ligand design.

Solvent Mapping/Probe mapping

Many approaches have evolved to characterize what will bind to a site since the original GRID (which uses a point probe[3]) and MCSS (which was the first example of using functional groups [24]). A number of groups[19,20] have developed variations on mixed solvent dynamics (reviewed in [25]), where simulations use as many as 16 different chemical probes to explore what could bind to (a usually flexible) binding site. In one ambitious study[26], the methods identified key interaction hot spots on an ensemble of structures derived from MD simulations of x-ray structures and homology models from which VS identified an FGF-23 antagonist. The optimized compound was subsequently demonstrated to have activity in mouse models.

A more specific example is the characterization of water molecules in protein structures. Programs like WaterMap[11] and MDMix[20] can identify which water molecules are energetically favorable to displace in compound optimization. One striking example is the identification of two high energy waters in the binding site of the FGFR kinase in the presence of a lead compound, which could be displaced by modification of the scaffold leading to Rogaratinib (BAY 1163877), a pan FGFR inhibitor[27]; another example is identification of Acetyl-CoA carboxylase inhibitors which are active in animal models of obesity and diabetes[28].

A variant on solvent mapping is hybrid structure- and ligand- based methods. One example is the ALTA-VS strategy[29], where pharmacophoric features are derived from the ligands. A virtual library of rigid fragments is constructed cutting all the rotatable bonds of the compounds in an available library and then computationally docking each fragment into the binding site, then evaluating energy of interaction (in this case using an MM-GBSA force field). The best scoring fragments can then be used as pharmacophoric points to direct VS. A more extreme example of this type of approach is where compounds validated as binding after VS against a homology model is used to refine the homology model for more comprehensive modelling[30].

Pose prediction and refinement

Once a binding pocket has been identified and characterized, the next step is to predict the binding pose of a ligand in that site – molecular docking. Assessing how well different programs can make this prediction has been a continuous industry for the molecular modelling community (see our own work with the program, rDock[31]). A recent paper[32] provides one of the more comprehensive studies of the past 5 years, assessing how well the experimentally observed pose can be predicted by ten different programs for a test set of 2002 protein-ligand crystal structures. There are two main criteria on which programs can be assessed: can the program generate the correct binding pose?, and does the scoring function successfully identify this pose as the most favored? In general, where receptor flexibility is not important, the correct pose can be generated and although for some protein families, there was reasonable correlation between docking scores and experimental binding affinities, the

ranking of the binding affinity was not well predicted across the whole dataset.

Success can be improved by combining docking with alternate scoring schemes. MM-GBSA can be used to rank order docking hits with some exciting examples[33-35]. A more recent development is dynamic approaches such as DUck[36] (Figure 1). DUck uses steered MD where a virtual "force" is applied to pull the ligand out of the binding site, while performing MD simulations to calculate the energetic cost of breaking a key hydrogen bond between a ligand and the protein. The theoretical relationship is tenuous, but this could be taken as related to the activation energy for the interaction, which will affect the binding energy. Another approach is free energy perturbation (introduced in [6]) with a number of examples[37-39].

Dynamic Docking

The evaluation of docking programs[32] usually tests for re-docking a ligand into the protein conformation obtained in the protein ligand crystal structure. What is more challenging (but the more realistic application scenario) is to predict the binding pose where there is some adjustment in the detailed conformation of the protein binding site. There are a number of approaches to address this problem. The first is to use different conformations of the protein as the target for docking. Ensembles of conformations for the protein can be generated by MD[40,41] or different experimental crystal structures can be used[42] as a project proceeds and more crystal structures obtained. One recent method development is a fast algorithm for sampling flexible protein-ligand conformations (known as PELE[43]) that was able to reproduce ligand induced side chain rearrangements and small main chain protein movement in a set of protein-ligand complexes, and performed better than MD simulations and induced fit docking.

A second approach is to account for receptor flexibility while docking and there have been a number of recent reviews[44-46] which survey progress. The methods rely on substantial computer power, so at present can only be applied to a few compounds and are more applicable for hit to lead optimization rather than hit identification. The methods fall into two main categories – those requiring an *a priori* definition of the pathway for conformational change and those that do not. In a recent publication[47] an induced fit docking protocol is used to generate possible conformational changes that enable ligand binding. These possible conformations are then assessed using metadynamics, a computational device to encourage a molecular simulation to explore across the possible conformations available, by introducing energy terms which discourage the simulation returning to conformations that have already been visited. Significant improvement in the quality of docking is reported across 42 test systems.

There are a number of recent publications where the simulation is unsteered and explores conformational change unsupervised. In one case[48], a technique called potential scaled MD was used to predict the binding pose in two test cases with conformational change. The method works by lowering the barrier between conformational states, in some ways emulating use of a high temperature. An alternate unsupervised method is adaptive electrostatic bias[49] where the electrostatic interactions are modulated depending on proximity of the ligand to the binding site – this

also reduces the barrier to conformational change. These new dynamics methods complement more conventional MD, as in the FGF example discussed above [26].

This is a promising new concept for which several retrospective examples have emerged - for example adaptive sampling allowed high throughput MD of a small fragment library for CXCL2[50], while there are even examples of long MD simulation used for *in silico* fragment screening[51], which also emphasizes the power of GPU-accelerated computational power in structure-based drug design.

Concluding remarks

The October 1st, 1981 edition of Fortune magazine heralded a "New Industrial Revolution" in which drugs can be designed by computer. The pharmaceutical industry has encountered waves of new technology (e.g. combinatorial chemistry, genomics). In most cases, it takes many years (decades) for the methods but also the expertise to develop so that the methods can make effective contributions to the drug discovery process. Structure-based, computational methods have suffered more than most new technologies in achieving this routine, productive phase. However, the techniques have now made recognizable contributions to the design of more than 50 compounds in clinical trials[52] and to several drugs on the market[53].

In this mini-review, we have focused on new developments in methods for just one aspect of SBDD – predicting how compounds bind to their target. What we have not discussed in detail is how these methods (combined with advances in predicting strength of protein-ligand binding) are now contributing to an increasing number of success stories where potent compounds are being identified for a range of targets. Looking back over the past forty years, perhaps it has not been an "industrial revolution", but more a continuous scientific evolution. Steady improvements in quality of the methods and understanding of how they can be used has led to increased acceptance and confidence so that the medicinal chemistry community now can appreciate how SBDD methods can contribute to the drug design process.

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Figure Legend
Methods for predicting ligand binding modes illustrated through an example of calculations for the kinase, CDK2 (protein structure taken from the PDB code: 1CKP). A) Binding site prediction by fpocket[15] (default settings) by clustering solvent inaccessible spheres and disregarding solvent exposed spheres. A "druggability" score is assigned to each predicted pocket. In this example, Site I, obtained the highest score (0.8), while the remaining eight pockets score very low (<0.1). B) Polar hot spots identified through mixed solvent MD simulations using MDMix[20]. Ethanol and water were used to probe the binding pocket, from which high and low energy areas are identified. The low energy areas probed by ethanol (deep purple), help to identify donor or acceptor features that could be exploited by ligand binding. Water (cyan) and hydrophobic (yellow) sites are also probed. C) These hot spots were then used to guide docking of the ligand from PDB structure 1PXM. Docking was performed

with rDock[31], using a donor as a pharmacophoric restraint (sphere) to interact with the backbone of LEU78 (yellow dashed line) in the CDK2 structure. D) This was followed by pose refinement using MD[54] to explore the flexibility of the pocket; for example, the yellow surface indicates possibility for a clash between ligand and protein.

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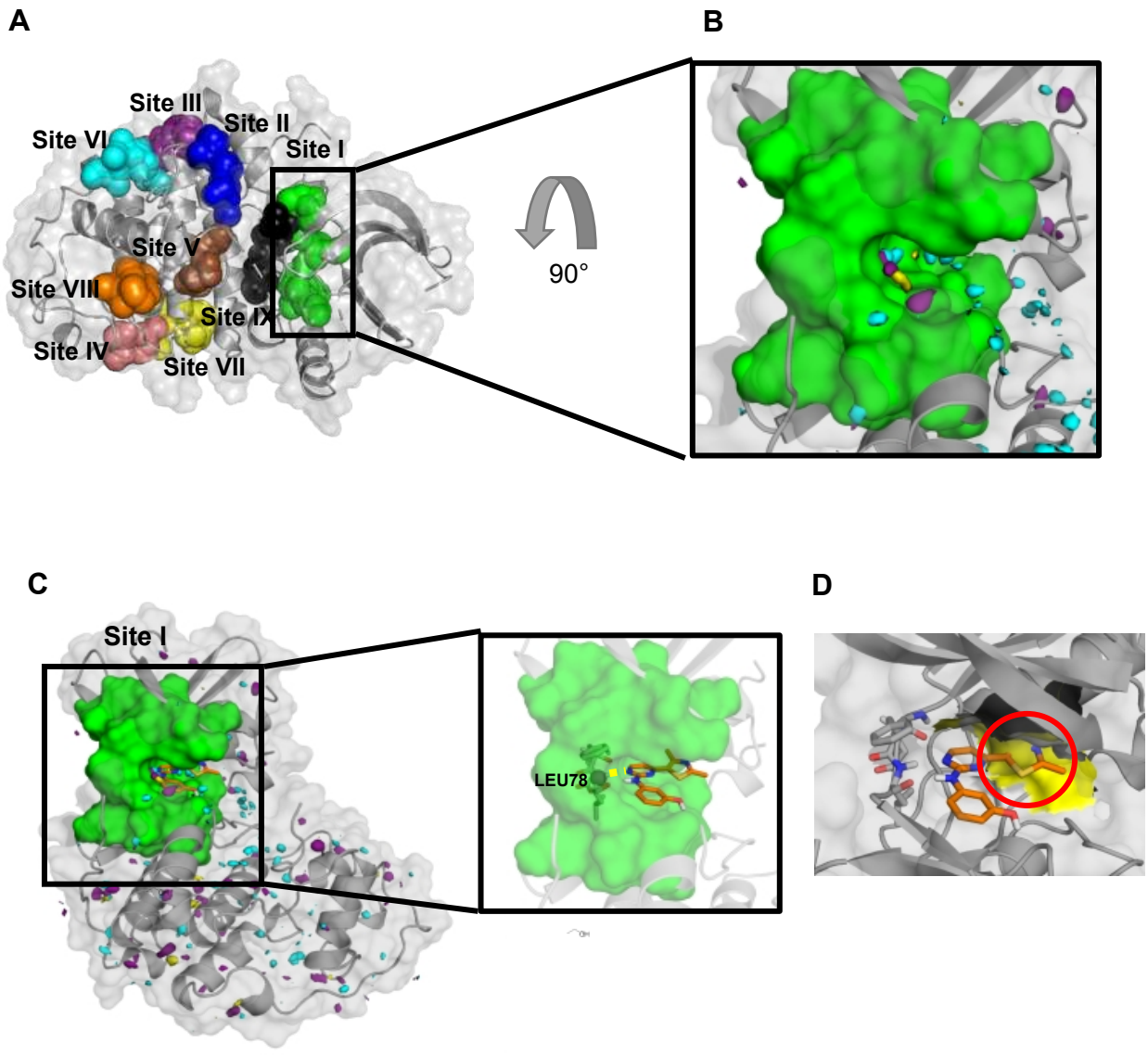
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