Molecular Modelling†

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1 Introduction

The enormous pressure that the pharmaceutical and biotech companies are facing, has created the need to apply all available techniques to decrease attrition rates, costs and the time to market. Currently, one of the most widely applied techniques in drug discovery is computational chemistry and molecular modelling. This branch of science is centred on applying the fundamental laws of physics and chemistry to the study of molecules. In the case of drug discovery, the molecules under study are those directly or indirectly involved in human disease. The ultimate aim is to create models and simulations, which can help in the different stages of a discovery pipeline by predicting, rationalizing and estimating the properties of molecules and their interactions, thereby allowing a more rational approach to drug development.[1] This whole trend is now seen both as an alternative and a complement to the more "brute-force" approach exemplified by the application of combinatorial chemistry and high-throughput screening (HTS).

The fundamental factor allowing the widespread use of molecular modelling is the central paradigm of today's drug discovery, the one-disease one-target concept and its implementation. Within this paradigm, a certain human condition is associated with the role played by a particular macromolecule, whose action can be modulated with a small organic molecule in order to achieve a therapeutic effect. With this perspective, drugs are developed in a sequential way. First, a macromolecular target to treat the pathology under study must be found, a process termed as target finding. Then, the search for small molecule binders (hits) for that particular target begins, the so-called hit finding stage. Once found, these binders must be optimized in order to achieve better *in vitro* activity, selectivity, pharmacodynamic and pharmacokinetic properties, the stage termed as hit to lead. Then, the lead must be optimized in a series of *in vivo* studies, the stage of lead optimization. Only when the lead has been optimized and tested in several animal models can the project then progress to human clinical trials. Computational chemistry and molecular modelling methods have become central features of all these pre-clinical research stages of the drug-discovery process.

When applied to the study of drugs and their receptors, molecular modelling techniques are generally divided into two broad categories. Ligand-based modelling consists of a series of techniques used for creating models and predictions based solely on the structure of the small organic compounds. In contrast, structure-based drug design (SBDD)[2,3] exploits the knowledge of the 3D structure of one or more biological receptors (targets, the ones sought to modulate and anti-targets, the ones sought not to interfere with) and/or their macromolecular ligands. These two broad categories are very often applied in a myriad of different combinations, so the frontier separating them is not clear-cut.

Molecular modelling as applied to SBDD has undergone a dramatic change over the last two decades. At first, the simulation of biochemical systems and their interactions was a nearly unfeasible task. The targeted macromolecules were treated in a very simplified way because of the great amounts of computation required. Often only a portion of the whole system could be dealt with, solvent effects were rarely taken into account, and the simulation of complex formation could only be carried out for a small number of molecules. This picture has changed dramatically in the last decade mainly due to two factors. First, as Moore's law stated in 1965, the number of transistors on a given chip has been doubling approximately every 2 years, with the subsequent impact on computer power. This has allowed an increase in the size of system that can be studied, the degree of accuracy of the models and the number of interactions feasible to calculate on a reasonable time scale. Second, there has been incredible progress in the experimental techniques that the different modelling tools rely on. X-ray crystallography and nuclear magnetic resonance (NMR) have been developed to a level where they are now applied routinely, which has had a tremendous impact on the number of experimentally determined molecular structures available. The number of both small molecules and macromolecules deposited in the Cambridge structural database[4] and the protein data bank (PDB),[5] respectively have increased dramatically. This wealth of experimental information has fuelled the refinement and application of many modelling tools, from force-field and scoring function development to homology modelling. The progress in the reliability of prediction, applicability of the different techniques and higher throughput capacity has enabled the application of structure-based molecular modelling in many phases of drug discovery, as will be reviewed below.

First, a brief outline of all the methods is presented, with references to the standard publications in each field. Then, a number of different applications are discussed and structured according to the usual progress of a drug-discovery project.

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2 Methods

2.1 Quantum Chemistry Methods

Quantum chemistry is the application of quantum mechanics (QM) to problems in chemistry. QM provides the most rigorous and physically meaningful description of molecular systems, most noticeably, the electrons are explicitly considered. In its purest form, QM is used to solve the wavefunction of molecular systems without any prior knowledge of the system or need for empirically derived parameters; hence its designation as *ab initio* methods. *Ab initio* methods have to rely on a series of approximations to provide a solution to Schroëdinger's equation and to speedup calculations, but the level of theory can be chosen to provide the best trade-off between quality of results and computational cost for each specific application. As *ab initio* methods are computationally very demanding and become prohibitive for relatively small systems (currently a few tens of atoms), semi-empirical approximations were introduced, which make use of experimentally determined data to avoid the calculation of certain terms, particularly two-electron integrals. Density-functional theory (DFT) provides a third class of QM methods; these methods are based on the fact that, for a system in its lowest energy state, there exists a one-to-one mapping between the electron density and the wavefunction of a system. As DFT methods give direct access to electron density, they are much faster than wavefunction-based methods of similar quality, although it is also true that they rely on some adjustable parameters. Each QM method has its own benefits and weaknesses, as well as a myriad of levels of theory to choose from. Moreover, a number of methods have been described to enable the extension of QM studies to large molecular systems.[6] These include further approximations that enable a better scaling of the computational cost with the number of atoms or the use of different levels of theory for different subsets of the system. It is not our intention to compare the many available options, as this has already been done in the literature.[7] Our aim here is just to outline the most common applications of QM methods to SBDD; hence, the use of QM methods in ligand-based applications[8] will not be discussed. First, we will introduce two types of applications that are well established, where QM provides a clear advantage over parametric methods, then we will present an emerging application that may become increasingly useful in the near future.

2.1.1 Ligand Internal Energy. Although biomolecules are generally too large to be described quantum mechanically, drug-like inhibitors are certainly amenable to QM methods, even at a high level of theory. In SBDD, the bioactive conformation of a ligand can either be experimentally observed or predicted based on the expected complementarity with the receptor. Very often this does not correspond to the absolute minima,[9] hence the internal energy of the ligand may play a major role in the structure–activity relationship (SAR) of a series. As molecular mechanics (MM)-based methods rely on general parameters, they often provide an unsatisfactory description of the internal energy of small molecules. As an example, it has been shown that approximately 10% of ligands adopt a bioactive conformation with an estimated strain energy of over 9 kcal/mol.[10] Clearly this is unrealistic, as it would imply losing over six orders of magnitude of binding affinity! Other instances, where determining the internal energy of the ligand may be crucial include tautomeric equilibrium, protonation states or barriers of conversion between isomers. QM methods can accurately calculate the energy associated with a configuration of a system, including non-equilibrium states and different topological arrangements, which MM-based methods cannot simulate properly. Calculations in the gas phase can be combined with methods to simulate the effect of solvation (*vide infra*), which can be indeed very useful for rational drug design. As an example, a recent set of calculations were used to identify the bioactive conformations of hypoxanthine and allopurinol, two substrates of the enzyme xanthine oxidase.[11] These modified nucleic bases present a large number of possible tautomers, which are very different in terms of their hydrogen bond donor/acceptor pattern. The authors used relatively high-level calculations, taking into account the effect of the solvent, to identify two tautomers for each molecule with very similar internal free energy in aqueous solution. Comparing the arrangement of donor and acceptor positions, they proposed a model of recognition of the substrates by xanthine oxidase.

2.1.2 Study of Reactivity. Unike MM methods, QM can simulate the breakage and formation of bonds; hence it can be used to study systems of pharmacological interest, where reactivity plays an important role. This includes inhibitors that react with their targets as well as the interaction of drugs with metabolic enzymes (*e.g.* cytochrome P450[12]). The study of the ligand on its own can be useful to obtain reactivity indices, which may correlate with the SAR for a series. Nevertheless, the local environment can influence reactivity very much and should, ideally, be considered. QM/MM methods[13,14] provide in that regard an optimal solution: the part of the system directly involved in the chemical transformation is treated quantum-mechanically, whereas the rest of the system is considered by means of a force-field, which makes it computationally accessible. As illustrated in a recent study of the hydroxylation of camphor by $P450_{cam}$,[15] with these methods it is possible to obtain a complete picture of the molecular recognition of the ligand by its receptor as well as of the reaction mechanism, including the source of enzymatic catalysis, identification of the transition states and intermediates, *etc*. The main limitation of these methods is that they require considerable simulation periods, and thus the QM treatment is generally limited to low (fast) levels of theory, which may compromise the quality of the results, particularly the estimation of the height of the reaction barrier.

2.1.3 Ligand–Receptor Interaction Energy. The huge size of biological macromolecules does not allow for a rigorous QM treatment of ligand–receptor complexes, but this can be achieved using hybrid QM/MM[16] or linear-scaling methods.[6] Within these approximations, the ligand can be described using a QM formalism, which in principle, should be more accurate than that obtained with parametric methods. The neglect of polarization and charge-transfer effects is an obvious limitation of the latter and thus, it received particular

attention in the first published studies where the interaction energy of inhibitors was considered by means of a QM/MM approach[17] or the divide and conquer method.[18] Nevertheless, these and other studies[19,20] failed to show a clear improvement of QM-based methods over the parametric ones for binding energy predictions. In a much more extensive study, Raha and Merz[21] use a semi-empirical description of the system to calculate the electrostatic term of the gas-phase ligand–receptor interaction energy and the electrostatic part of the solvation free energy. These terms are then combined with the attractive part of the Lennard–Jones interaction potential and approximated conformational and solvent entropy terms to obtain an estimate of the ligand–receptor binding free energy in solution. The authors show that this scheme can be very useful to predict binding free energies and to discriminate between native and decoy poses (*i.e.*, proposed binding modes) of ligands. Although the results of these recent studies do not suggest a major leap in terms of quality, considering that these methods require far fewer parameters than purely parametric methods and that they are capable of capturing non-pairwise additive effects, a great deal of attention should be paid to QM-based scoring methods.

2.2 Parametric Methods

The forces that govern the structure of molecules, both in gas and condensed phase, are generally well known and can be used to rationalize and predict the behaviour of molecules. This enables us to partition a whole system into smaller units that interact with each other following certain rules. A very successful and widely applied approach in molecular simulation consists of using a set of molecules to derive rules and parameters of more general applicability. These are known as parametric methods, which we have further divided into three major classes based on the approach used to obtain the parameters and the scope of the model. The first class is force-fields, which aim at providing a complete description of the system. The other two classes of parametric methods presented here are only concerned with the process of molecular recognition.

As we will show in the next sections, parametric methods play a major role in computational simulation of biological systems, but equally important for drug discovery is the fact that chemists, in general, and molecular modellers, in particular, have a good working knowledge of the physical forces behind molecular recognition (mainly electrostatic, van der Waals, hydrogen bond, π-aromatic and hydrophobic).[22] This enables them to assess automatic predictions, generate hypotheses and make timely decisions.

2.2.1 Force-Fields. Within the Born–Openheimer approximation, the movement of electrons and nuclei can be considered independently; hence it is possible to study only the movement of the atomic centres assuming that the electron distribution is always in equilibrium. Molecular force-fields make use of this approximation to bypass the calculation of such electronic distribution and replace it with functions and parameter sets that describe its effects. As a result, molecules and atoms become classical particles, whose mutual interactions are governed by bonded and non-bonded terms that adopt simple forms (see Equation (1)); hence the name of MM. The parameters describing the force-field are derived from experimental data and/or high-level QM results. The most common and well-validated force-fields for biological macromolecules are AMBER,[23] CHARMM[24] and GROMOS,[25] while force-fields such as OPLS[26] or MMFF94[27] were designed to be as general as possible and are widely used to simulate small molecules. In spite of the crudeness of the approximation, MM-methods have a long history of success and have become a fundamental tool not only for computational chemistry but also for structural sciences.[28] Some 'classic' docking packages, particularly DOCK (see later) use force-fields as scoring functions. One potential issue with this approach is that in order to rapidly obtain parameters for the ligands, the calculation of partial charges relies on fast but inaccurate methods based on electronegativity indices[29] instead of QM methods such as those used in force-field development.

$$\begin{aligned} V &= V_{\text{bonded}} + V_{\text{nonb}} \\ V_{\text{bonded}} &= \sum\nolimits_{\text{bonds}} K_b(d-d_0) + \sum\nolimits_{\text{angles}} K_a(\theta-\theta_0) \\ &\quad + \frac{1}{2}\sum_{\text{dihedrals}} K_d(1+\cos(m\phi-\gamma) \\ V_{\text{nonb}} &= \sum_{\text{nonb}} \left(\frac{A}{r^{12}} - \frac{B}{r^6} + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}\right) \end{aligned} \tag{1}$$

2.2.2 Empirical Scoring Functions. Empirical scoring functions estimate the binding energy of a ligand conformation in terms of physicochemical interactions such as hydrogen bonding, ionic and hydrophobic interactions, calibrated against complexes of known affinity. Most of the empirical scores in use (*e.g.* FlexX, [30] ChemScore[31]) today derive ultimately from the pioneering work of Bohm,[32,33] as incorporated in the LUDI program.[34] Empirical functions generally perform well in binding mode prediction and hit identification (enrichment), but are less successful at accurately ranking active molecules by binding free energy. Pure empirical scoring functions have been combined with van der Waals terms from molecular force-fields to produce the so-called semi-empirical scoring functions (*e.g.* GOLD,[35] LigScore[36]).

$$\begin{aligned} \Delta G_{\text{bind}} = \Delta G_0 + \Delta G_{\text{hb}} \sum\nolimits_{\text{h-bonds}} f(\Delta d) f(\Delta\alpha) + \\ \Delta G_{\text{hb}} \sum\nolimits_{\text{ionic}} f(\Delta d) f(\Delta\alpha) + \Delta G_{\text{lipo}} A_{\text{lipo}} + \\ \Delta G_{\text{aro}} \Delta N_{\text{aro}} + \Delta G_{\text{rot}} NR \end{aligned} \tag{2}$$

2.2.3 Statistical Potentials. The third category is that of knowledge-based statistical potentials, exemplified by potentials of mean force (PMFs). The principle is that the observed distribution of distances between pairs of different atom types is a reflection of their energy of interaction. In practise, large training sets of protein–ligand structures are analyzed to provide sets of distribution functions. These are then converted to sets of atom-pair potentials using the inverse Boltzmann

technique, which provides an energy value for a given state based on observed probabilities; no experimental binding affinities are, thus, needed. Examples of protein–ligand potentials include BLEEP,[37,38] PLP,[39] PMF Score[40] and Drug Score.[41] The various approaches differ in the sets of protein–ligand complexes used to obtain these potentials, the form of the energy function, the definition of protein and ligand atom types, the definition of reference states, distance cutoffs and several other parameters. In addition to scoring protein–ligand complexes, these potentials have been used to evaluate protein–protein complexes[42] and in protein structure prediction.[43] For docking applications, PMFs are generally not used during the optimization phase but mostly to identify decoys or to use in combination with other scoring functions in virtual screening (VS) applications (consensus scoring).

$$\Delta G_{\text{bind}} = \sum_{\text{A}} \sum_{\text{B}} \text{U}_{\text{AB}}(r_{\text{AB}}) \quad (3)$$

2.3 Solvation

Water has a strong influence in all biochemical phenomena, and specifically plays a central role in molecular recognition. It profoundly alters properties such as the dipole moment and the molecular electrostatic potential, affects the conformational and tautomeric preferences of both small molecules and their macromolecular targets, and governs the hydrophobic effect, by which non-polar molecules (or the non-polar parts) tend to aggregate to reduce the solvent exposed hydrophobic surface therefore minimizing the loss of entropy associated with the ordering of water molecules.[44,45] Because ligand–receptor non-covalent association takes place in an aqueous environment, the role of water must be taken into account in order to qualitatively understand this process and also quantitatively determine its free energy.

The effect of water can be introduced in different ways depending on the representation of the system (solute) under study. When the system is represented as a quantum mechanical particle, the effect of solvent can be introduced explicitly or implicitly. However, because of computational limitations, the former is seldom used and virtually always the latter is preferred. The most popular methods to account for solvent effects are the continuum methods, where these effects are introduced as a perturbational operator representing the solvent reaction field. Because the solute wavefunction and the reaction field depend on each other, they have to be solved using a self-consistent procedure. Many quantum mechanical continuum methods have been developed, varying basically on the definition of the solute/solvent boundary and the description of the reaction field and the solute charge distribution (see reviews by Cramer and Trular[45] and Orozco and Luque[44]).

When the system is described by a classical model based on a force-field, the simplest way of taking into account the effect of water is representing it discretely. If velocities are given to such a system, as in a molecular dynamics (MD) trajectory, a solvated ensemble of the molecular system is obtained, where the atomic coordinates of both solvent and solute are produced. This provides an insight into the differential solvation of certain parts of the solute, generates radial distribution functions, and allows an assessment of whether water molecules bridge ligand and receptor, *etc*. When coupled to statistical mechanics, it can also be used to calculate differential free energies of solvation between different solutes with the help of thermodynamic cycles.[46] However, one of the most serious drawbacks of the explicit water treatment is its computational expense.

Less computationally demanding methods have been developed to account for solvent effect on a classical system. Among the most popular are the classical continuum electrostatic methods, where the solvent is treated as a continuum environment. The solute molecule is placed in a cavity, whose permittivity usually ranges from one to eight, and surrounded by a polarizable continuum medium with a defined solvent dielectric constant. The main difference among the classical continuum electrostatic methods is in the definition of the solute/solvent boundary and how the solute–solvent electrostatic interaction energy is calculated. The most popular methods are the Poisson, or Poisson–Boltzmann (PB)[47] if the effect of counterions is also taken into account, and the Generalized Born (GB) model.[48]

Other implicit water-treatment methods for a classical system are those derived empirically. Although less rigorous, they are still used because of their low computational cost. A first group of methods makes use of parameters for modelling screening of electrostatic interactions by water, replacing the macroscopic permittivity by a distance-dependent dielectric function. In the simplest models the latter can change linearly,[49] but more complex models where it changes exponentially have also been developed.[50] A second group of empirical methods is based on the solvent accessible surface area (SASA). In them, it is assumed that solvation free energy can be calculated by addition of the contribution of each atom or group of atoms. Each atom type is given a solvation parameter obtained by a fitting procedure and the contribution of each atom is based on its SASA.[51]

2.4 Sampling Algorithms

Once the chemical system under study has been defined and any necessary parameters have been obtained, one can proceed to run calculations on it. Usually flexible molecules have a complicated potential energy surface, with several minima and saddle points, which are a function of the nuclear coordinates. Especially interesting are the configurations that correspond to minima in the potential hypersurface as these are stable states of the system. The identification of these minima will generally consist of two steps: global exploration and local minimization. Although it is theoretically possible to systematically explore each degree of freedom, in practice, this can only be done in a reasonable time frame for very small systems; hence, stochastic methods are most commonly used in SBDD. Evolutionary computational techniques such as genetic algorithms (GA) are particularly widespread. These methods start by generating a random collection of candidate solutions whose fitness is evaluated, the best individuals are then stochastically selected and mutated or recombined to obtain a new population. This process is then repeated until a certain convergence criterion is achieved.

For local optimizations, there are several minimization algorithms that search the nearest minimum in the potential surface. These can be broadly classified into two groups, those that do not use the derivatives of the potential energy with respect to the coordinates, such as the simplex method, and those that do, such as the steepest descent and conjugate gradient methods. The latter operate in an iterative procedure: (1) potential energy evaluation for a given configuration; (2) determination of the first (gradient) and second derivative of the energy with respect to the coordinates; (3) generation of a new set of coordinates in the direction of the minimum and (4) energy evaluation for the new set of coordinates. If the energy is converged the calculation stops, if not, the process is repeated again. They are often used in SBDD in several contexts, such as the initial refinement of a protein structure obtained by experimental methods or to relax the geometry and eliminate unfavourable contacts of a ligand–receptor complex.

However, the biggest limitation of a minimized molecular system is its static character. Molecules vibrate and constantly change conformation, overcoming potential energy barriers and populating an ensemble of microstates, which are globally responsible for the properties of the system. To generate such an ensemble different algorithms can be used, the most widely used of which are MD and Monte Carlo (MC).

MD is based on the application of Newton's equations of motion to describe the evolution of a classical system along time. When the system is defined with a force field, it is feasible to calculate the forces acting upon each particle (atom) by obtaining the gradient of the potential energy. Once the force on each particle is known, its acceleration can be derived, which by integration determines the velocity and position after a time increment. After generating the new set of coordinates the steps can be repeated again in an iterative fashion. The result is a set of structures that represent the evolution of the system along a time path. The only prerequisites for the calculation of a trajectory are a set of initial coordinates, as the initial velocities are randomly generated. Calculation of a trajectory develops a dynamic view of the molecular system to have an ensemble that can later on be used to calculate free energies of binding for a ligand–receptor complex[52] (see below).

Restrained MD is a special case used extensively in the refinement of macromolecular structures with data derived from experimental techniques. In restrained MD, additional terms are added, which complement the original potential energy definition of the system. These terms do not have a chemical sense, but penalize those conformations that do not respect the experimental data. Thus, for instance, an NMR experiment can provide interproton distances that can be added as an extra retraint function to the original force-field in a similar way as a stretching function defines a bond between two covalently attached atoms. In this way, only the structures complying with the combined potential are allowed. This strategy is nowadays routinely used in the refinement of structures by NMR. In a similar way, distance and dihedral restraints can be derived from the alignment of two protein sequences and also added to the original force-field definition; this is a central strategy used in homology modelling, which will be presented below.

While in MD, there is a time dependency, MC also generates an ensemble of states, but in a stochastic fashion. Starting from a given conformation, a perturbation is introduced to the system by modifying a random degree of freedom by a random small quantity. Then a ratio of probabilities is computed for the trial and original configurations and, from this quantity, a decision is made to accept or reject the trial configuration. Usually, in molecular simulations, the metropolis criterion is used to decide whether the trial configuration is accepted or rejected. First, the energy change (ΔU) of the system is measured on introduction of a perturbation. If the trial configuration has lower energy than the original, it is accepted; otherwise a function of probability (ω) is calculated,

$$\omega = \exp(-\beta \Delta U$$

where β is a factor that depends on the temperature at which the simulation is carried out. Finally, a random number (r) between 0 and 1 is generated and the trial conformation is accepted if $r < \omega$. Thus, the probability of accepting a new configuration is greater if the increase in energy is small or if the temperature is high. The efficiency of MC greatly depends on the moves that are tried and is important to choose perturbations that explore significantly different configurations but also provide a relatively good acceptance ratio (in the region of 50%).

MD and MC as sampling algorithms are crucial in the application of statistical mechanics methods, which are considered as the most rigorous classical methods for the determination of changes in free energy of binding for a ligand–receptor complex.[46] The two most frequently used methods are free energy perturbation (FEP) and thermodynamic integration (TI). These two techniques estimate free energy changes or $\Delta\Delta G$ between two inhibitors, thanks to the use of thermodynamic cycles.[46] A perturbation is used to smoothly convert one ligand A to another ligand B with the help of a coupling parameter both in solvent and within the protein environment (see Figure 1). Because the $\Delta G_{\text{binding}}$ A and $\Delta G_{\text{binding}}$ B cannot be obtained in a simulation, the differential free energy of binding between both molecules must be obtained by solving the other two terms in the cycle, $\Delta G1$ and $\Delta G2$, which can be calculated in an MD or MC simulation. By subtracting both of these values, one can have an estimation of the changes in affinity between molecules A and B for the same receptor.

3 Applications

3.1 Target Evaluation

Computational chemistry typically becomes associated with a drug-discovery project at the hit-identification stage, but it is important for the modeller to understand and make a thorough assessment of the tractability of the chosen biological target, as well as the quality of the structural information. In extreme cases this may lead to the suggestion of abandoning a target or to pursue a non-structure-based strategy. The real goal of this stage is, nevertheless, to make a rational and optimal use of the available information, to identify possible pitfalls and to start a project with the greatest chance of success.

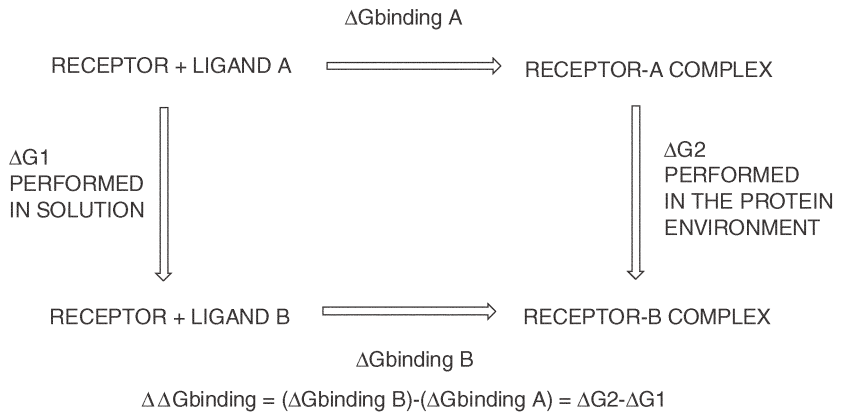


Fig. 1 Thermodynamic cycle used to compute differences in free energy of binding between two inhibitors, A and B

3.1.1 Target Druggability. Once the link between macromolecule and human disease has been discovered and validated *via* several experimental techniques such as gene silencing, knockout mice and animal disease models, the most critical question arises. Is the target druggable? That is, can it be modulated with high affinity and selectivity by a drug-like organic molecule delivered by an oral route? Retrospective analysis of marketed oral drugs and those that fail in the different stages of drug discovery have allowed the definition of some general trends that distinguish between drug-like and non-drug-like molecules, and allowed the setting of boundaries to the so-called drug-like chemical space.[53] One such rule is the famous Lipinki's rule of 5,[54] which predicts high probability of failure due to non-drug-likeness for molecules not complying with 2 or more of the following rules: (i) molecular weight below 500 Da; (ii) a calculated Log*P* less than 5; (iii) less than 5 hydrogen bond donors; (iv) oxygen + nitrogen count of less than 10. Additional analyses have shown the importance of rotatable bond count, which should be less than 10, and polar surface area (PSA), with a threshold of 140 A^2, for the bioavailability of a compound administered orally.[55] Only the macromolecules that can be acted upon by organic molecules meeting all these requirements are considered druggable. A recent analysis estimated that out of the total number of genes in the human genome (around 30,000), only 3,000 might code druggable proteins.[56] Remarkably, only around 400 of such targets have been studied so far,[56] accounting for 13% of the total number estimated.

Although empirical, all these limits to chemical space come from the selection pressure enforced by the functioning of the human body, and restrict the number of macromolecules that can be treated effectively with the traditional medicinal chemistry approach. Thus, although DNA could in principle be the perfect target, modulation with high potency and selectivity of a unique stretch of double stranded DNA would require a chemical agent well outside drug-like chemical space. The sheer size and number of hydrogen bond donors and acceptors that a potential drug would need to bind selectively and potently to the minor groove of a DNA duplex would exceed by far the drug-like property limits. Therefore, among the four types of macromolecules found in the human body, carbohydrates, lipids, nucleic acids and proteins, only the latter usually make druggable targets. However, not all proteins directly linked to a specific disease are druggable. Indeed, an analysis of marketed small molecule drug targets reveals that more than three quarters are enzymes and membrane receptors, proteins which usually bind small molecule endogenous ligands in a well-defined and secluded cavity,[56] thus making it possible for a small chemical agent to compete with them and exert a therapeutic effect. This implies that a good portion of the human proteome might not be druggable even if closely related to human pathology.

The most relevant application of structure-based molecular modelling at the initial stage is finding the important areas of the receptors that make good binding spots for a chemical agent (see Table 1). Usually this involves detecting the active site for enzymes, membrane and nuclear receptors, where their endogenous ligands bind, as well as finding the crucial epitopes and clefts involved in many of the protein–protein interaction partners known from proteomics studies.[57] Thus, for protein–protein contacts it involves finding those particular ''hotspots'' (in the usually huge contact surface), which contribute the most to the non-covalent association of both proteins.

For active site detection, many algorithms have been devised to highlight the possible small molecule binding points. One of the earliest attempts was developed by Goodford[58] and relies on the calculation of interaction energies. Typically, the protein structure is immersed into a cubic grid, and the interaction potential between receptor and a number of different probe particles placed at each grid point is calculated (probes can be charged atoms, a water molecule, or a hydrophobic particle, *etc.*). The result can be visualized graphically in a series of contours of different energy values, which give a ''feel'' of where certain chemical groups better interact with the receptor. Because active sites are small, sterically limited and usually hydrophobic cavities, the contours help to highlight the energetically favourable sites. Alternatives to the interaction energy approach have been developed, which rely on purely geometric methods.[59] An example is the Site Finder program implemented in molecular operating environment (MOE),[60] which locates binding sites by first calculating empty spheres contacting four protein atoms on their boundary. The spheres that correspond to inaccessible sites of the protein, as well as those that are too

Table 1 Parameters to consider in assessing the druggability of a binding site

Parameter	*Ideal value*	*Explanation*
Shape	Deep or enclosed	The ligand–receptor interaction energy roughly correlates with the surface-contact area. Small molecules require enveloping cavities to attain sufficient binding affinity.
Size	Fits ligands of 300–600 Da	Small cavities may not be able to accommodate drug-like molecules. Very large cavities may not provide sufficient surface-contact area.
Chemical character	Mix of hydrophobic and hydrophilic.	Drug-like molecules present a balance between lipophilicity (low log*P*) and hydrophilicity (H-bond donor/acceptors, PSA).
Flexibility	Rigid	Binding to very flexible binding sites involves an entropic penalty. Flexibility of the receptor is a difficult property to model.

exposed to solvent are eliminated, effectively leaving those that correspond to regions of tight atomic packing. The spheres are then classified as hydrophobic or hydrophilic and all those hydrophilic spheres not close to at least one hydrophobic sphere are eliminated. Finally, the spheres are clustered giving a collection of sites, which are ranked according to the number of hydrophobic contacts to the receptor. This simple technique can be efficiently used for active site detection. Parallel to this, other efforts are being directed at compiling structural information on all the active sites solved by experimental methods (*e.g.* the catalytic site atlas[61]). These databases are and will increasingly be very helpful for active site detection and even for catalytic function prediction, irrespective of the level of sequence identity of the proteins under study.

The detection of protein–protein interfaces is a great challenge for structure-based *in silico* techniques. In contrast to active sites, these interfaces are huge and shallow surfaces, barely distinguishable from other parts of the protein. The detection of the cluster of residues that contribute the most to binding (hot spot) within these interfaces is an even bigger challenge. A promising approach to detect the binding interface for non-obligate protein complexes has recently been reported where the interfaces are highlighted by predicting the optimal docking area (ODA) of a protein.[62] The method identifies area patches with optimal docking desolvation energies using a simple accessible-surface-area (ASA) method, and it is reported to have an 80% success rate. Also recently, an extensive analysis of protein interfaces in the PDB has shown that binding interfaces can generally be detected by analysing structural (not necessarily sequential) conservation of certain aminoacids within protein families, specifically tryptophan, and less pronouncedly phenylalanine and methionine.[63] These results are reinforced by an independent analysis of protein–protein pairs in which it was found that the core surface of the interface is enriched in tryptophan, tyrosine, methionine and phenylalanine residues.[64] Although these methods discriminate binding interfaces from the rest of the protein, the detection of the epitopes within them that can be mimicked by a small molecule is very complicated, and will likely require a combination of *in silico* and experimental techniques, as has been the case in the development of VLA-4 integrin antagonists.[65–67] Finally, it should be emphasized that antagonizing a protein–protein interaction is still perceived to be difficult and risky by the drug-discovery community.[57] Usually the molecules showing promising *in vitro* activity tend to have molecular weight, hydrogen bond donor and acceptor counts on the limit of what is considered desirable, as can be seen for instance in the development of IL-2 and integrin antagonists.[57,68]

3.1.2 Structure Availability and Critical Assessment. The availability of structural information of a macromolecular target opens the door to SBDD. Thanks to the progress in structure determination methods and the structural genomics initiatives it is ever more common to know the structure of the target, yet certain protein families, such as G-protein coupled receptors (GPCRs), remain formidably challenging.[69] Although the structure of macromolecules can be solved in an increasingly automated way both by X-ray crystallography[70] and NMR,[71] an inspection of Table 2 clearly indicates that the latter is seldom used to obtain the structure of ligand–receptor complexes. This, combined with the fact that usually there is less uncertainty associated with X-ray than NMR structures, explains why the vast majority of experimental structures used in SBDD projects originate from the former technique. These structures are, nevertheless, just models limited by the nature and quality of the experimental data, hence a few rules are provided here to enable the end user of crystallographic information to make a critical assessment of the structures prior to using them.

Even with the fast progress in high throughput protein crystallization,[72] it will take some years before experimental structures are available for all proteins of potential pharmacological interest. However, the number of possible folds is significantly lower and structures currently available already represent most of the protein families,[73] and computational methods to generate 3D models based on homology are increasingly accurate and can be used in SBDD. These will be presented next.

3.1.2.1 Considerations regarding the use of crystallographic structures. As the structure of a target underpins all computational SBDD methods, a good understanding of how the structure is obtained and its potential limitations is not only useful, but really necessary to avoid misinterpretation or loss of information. An excellent recent review has analysed in detail the use of crystallography in drug design and the

Table 2 Contents of the PDB as of May 2005. Ligands are defined as heteroatom records not containing metals, with more than 10 heavy atoms and a molecular weight between 150 and 800 Da

	No. of PDB entries			*No. of unique ligands bound to proteins*
	All	*Proteins*	*Protein-ligand complexes*	
X-ray	26255 (85%)	24301 (87%)	9895 (99%)	3362 (99%)
NMR	4548 (15%)	3776 (13%)	132 (1%)	73 (2%)
Total	30803	28077	10027	3395

limitations of the method.[74] Here we will only outline the most important factors that one should consider, as listed in Table 3. The resolution of the data (expressed in Å) and the R- and R free-values provide an idea of the overall quality of the structure as a whole, whereas B factors (or temperature factors) indicate the reliability of individual atom positions. In the absence of electron density maps (which may sometimes be available[75]), these parameters are useful to get an idea of the experimental uncertainty associated with the coordinates. The PDB files often contain some information that can be easily overlooked, such as comments in the Remark section or multiple positions of certain atoms due to experimental double occupancies. For this reason a careful examination of the PDB files and additional information (*e.g.* accompanying papers) is strongly recommended. Depending on the quality of the crystal and the flexibility of the system, certain parts may not have observable electron density. If this affects the targeted site, the model will have to be completed prior to use. The number of electrons also plays an important role: while the coordinates of hydrogen atoms are almost invariably missing, the positions of heavy atoms (S, P, Cl, Br, *etc.*) can usually be assigned with much more confidence than first-row elements. Even when the electron density is very clear, it is not possible to distinguish between isoelectronic groups (see Table 3 for a list). For these atoms, the assignment will reflect, at best, the personal interpretation of the crystallographer based on the interactions that the group makes with its surroundings. Finally, crystallization itself is another potential source of error: as the protein is in an environment sensibly different from solution, the structure can be affected by packing or the crystallization solution; furthermore, all the proteins in the crystal are generally considered identical, meaning that heterogeneity is ignored.[76]

Table 3 Considerations regarding the use of crystallographic data

Interpretation of PDB files	Resolution
	R-value and R free-value
	B factors
	Partial occupancies
Electron density-related issues	Isoelectronic groups:
	Proteins: Asn, Gln, His side chains
	Electronically symmetric ligands or ligand moieties
	Solvent/Ions: $H_2O = Na^+ = NH_4^+$
	Poor or lack of observed density:
	Side chains, particularly of flexible polar residues
	Mobile loops
	Domains
	Solvent molecules can be missing or confused with noise
	Hydrogen atoms not observed:
	Undetermined tautomeric and protonation states (*e.g.* His)
	Orientation of rotatable hydrogens (*e.g.* hydroxyl, water)
	If data is poor, the model will rely on dictionary parameters, which may be wrong for ligands.[78]
Crystal	Crystallization conditions: pH, salt concentrations, *etc*
	Packing effects
	Heterogeneity neglected

In an ideal scenario, the modeller will work closely with the structural scientists, enabling them to provide mutual feedback during the structure generation process and the modeller to receive much more than a set of coordinates. This is fundamental if high-throughput crystallography[77] has to translate into high-output in drug discovery.

3.1.2.2 Homology modelling. The sequencing of entire genomes in recent years has produced many more sequences than the structural genomics initiatives can absorb. In addition, some proteins can be very difficult to crystallize, even if close homologues crystallize well, and may require substantial time and efforts to obtain a structure.[79] A faster alternative is to generate a theoretical 3D model of the protein. *Ab initio* prediction of the structure of a protein is a formidable problem and, in spite of advances in protein folding studies,[80] the most reliable method to predict the structure of a protein is by comparison with related proteins for which the structure is known, a technique known as comparative or homology modelling.[81] A common first step in all homology modelling tools is to align the sequence that has to be modelled (query protein) with sequences of proteins of known structure (templates). The identity between these sequences provides a first indication of the reliability of the model; as a rule of thumb, if the level of identity is lower than 30% then the result of the alignment may be dubious and any model based on it questionable.[82] The sequence alignment allows generation of an initial 3D model of the query sequence, which can be restrained to certain coordinates or dihedral values of the reference structure to produce a refined model by restrained MD.[83]

3.2 Hit Finding

The discovery of chemical entities with a desired biological activity is the first milestone in the quest to obtain a drug candidate. The activity of interest can only be identified with a relevant biological assay, but computational methods can be used to identify chemical structures with a greater probability of being active. This process is often referred to as VS or screening of virtual libraries. Docking is, by far, the structure-based method most commonly used in VS; *de novo* design provides an interesting alternative. Both methods, and particularly its application to VS, will be described here.

Docking and *de novo* design methods are, nevertheless, seldom used as stand-alone tools for VS. This is because a wealth of experimental information from very different sources is available in a pharmaceutical research project and optimal results are obtained when several complementary methods are combined together. The integrative aspects of structure-based VS are, therefore, most important and merit particular attention. In this section, we will also consider two special cases of hit finding: template and scaffold hopping.

3.2.1 Docking. Molecular docking was first applied to drug design more than 20 years ago[84] as a computational tool that combines a search algorithm to generate putative binding modes of a ligand into its receptor with a scoring function that ranks them. Although the basic principles remain the same, many new algorithms and scoring functions have been developed and continue to be developed. A detailed survey of the progress in the field has been presented in recent reviews.[85,86] The main considerations regarding docking software are the scoring function(s) and search algorithm(s) that it uses (*vide supra*) and to which extent the flexibility of the ligand and the receptor are considered. This is summarized in Table 4.

The early realization that the conformation of small molecules in complex with macromolecules does not generally correspond to a global minimum[9,10] and that proteins undergo structural rearrangements upon binding of ligands[92] highlighted the necessity to incorporate flexibility in docking algorithms. This would represent a major burden for the search algorithms because, in addition to the rotational and translational degrees of freedom of the ligand, they would have to consider the fluctuations of bond distances and angles as well as torsions. Considering the size and flexibility of macromolecular receptors and the time constraints applied to docking, this is not really feasible. At present, most docking applications consider the receptor as a rigid body and, on the ligand side, only the degrees of freedom corresponding to dihedral angles are explored, either during docking (flexible docking) or by means of pre-generated libraries of conformers (rigid docking). This consensus should enable direct comparison of different docking packages and/or protocols; nevertheless, this has traditionally been difficult and had to rely on data published by different authors, often using different test sets.[93]

Table 4 Main aspects of docking engines

Item	*Options*	*Examples*
Scoring function	Force-field	DOCK
	Empirical	LUDI, FlexX, AutoDock
	Semi-empirical	GOLD
	Knowledge based	—
Search algorithm	Systematic	—
	Stochastic: GA	GOLD, AutoDock
	Stochastic: MC	ICM, Glide
	Stochastic: Other	PRO_LEADS (Tabu)
	Deterministic (*e.g.* simplex minimizer)	—
Ligand flexibility	None or implicit (precomputed conformers)	All
	Incremental construction	FlexX, DOCK, Glide, Surflex
	Full flexibility	GOLD
Receptor flexibility	None or implicit (multiple cavities)	All
	Implicit (“soft” docking)	Most
	Terminal polar hydrogens	GOLD
	Water molecules	—
	Side chains	SLIDE
	Grid averages[87,88]	DOCK, AutoDock
	Unified receptor description[89,90]	FlexE, DOCK
	Full flexibility of the binding site	ICM[91]

Note: Please note that this is not intended as an exhaustive list and that docking packages may include several features of each class.

Very recently, a profusion of studies comparing the performance of docking tools have been published.[94–103] In most cases, the comparisons are made by groups not involved in the development of the evaluated software. Two main metrics have been developed to characterize docking performance. The first is predicting the position and conformation of a ligand (the binding mode) for a known protein–ligand complex structure. The second is to calculate the enrichment factors that can be obtained in VS against a particular receptor protein. Here, a large library of varied organic molecules is seeded with compounds that are known for binding to the receptor. VS orders the library of compounds on the docking score to the receptor. The enrichment factor (EF) is calculated as how many of the known compounds are found on the top 1% of this list, compared to random. Although it is still difficult to rank the docking tools according to their performance, a number of trends have emerged.

Most docking programs will correctly predict the binding mode for 70–80% of the protein–ligand pairs within an RMSD of 2 Å

The programs providing best results in binding mode prediction are also best in VS experiments.

Programs with empirical scoring functions that have benefited from large and diverse validation sets currently available tend to provide the best results (*e.g.* Glide, GOLD and Surflex).

The results are largely receptor dependent. Some programs are more consistent than others (*e.g.* Glide).

Runtimes can vary widely; some programs (*e.g.* FRED) have specifically been developed for massive screening.

Overall, many docking tools have proved their usefulness in controlled VS experiments and their performance is expected to improve because this is a very competitive area, which is under very active development and receives considerable attention both from companies and academic groups (see Table 5). The published comparative studies have also played a fundamental role in identifying and making large and diverse test sets publicly available, which will facilitate further

Table 5 Available docking software cited in 10 recent comparison studies[94–103]

Program	*Developer*	*Citations (out of 10)*
DOCK	UCSF	8
GOLD	CCDC	8
FlexX	Tripos	7
GLIDE	Schrödinger	6
ICM	MolSoft LLC	3
Ligand fit	Accelrys	3
FRED	OpenEye	2
QXP (FLO+)	Thistlesoft	2
AutoDock	Scripps	1
DOCKVISION	DockVision Inc	1
SLIDE	Michgan State U.	1
SURFLEX	Discovery Partners Int.	1

comparisons and encourage the developers to thoroughly validate their docking software.

The two main areas that are expected to receive most attention in the near future are: (i) improvement of the docking scoring functions and (ii) introducing the flexibility of the receptor in docking applications. The first includes further optimization of empirical and knowledge-based scoring functions, driven by the incessant increase of available experimental data,[104,105] as well as more rigorous physical-based scoring functions, which should consider polarization and/or solvation effects.[21] The excellent results obtained with consensus scoring[106] suggest that most docking packages will implement several scoring schemes to facilitate its application. Regarding the flexibility of the receptor, a number of recent studies have shown that VS results can improve when it is accounted for either implicitly[107] or explicitly.[89,108] Nevertheless, it has been recently shown that flexible receptor docking is very prone to generating false positives and it should be cautiously applied to VS.[109]

Docking has been widely applied to hit discovery with remarkable success,[93,110,111] but the benefits of VS can only be properly assessed when compared with random screening. Unfortunately, head-to-head comparisons of HTS and docking-based VS are very rare. Merck researchers screened two different subsets of the corporate collection against dihydropicolinate reductase; the first subset was screened whole, while the second was pre-filtered with the docking program FLOG;[112] the corresponding hit rates were $\leqslant$0.2% and 6% resulting in a $\sim$30-fold improvement.[113] A second comparison was provided by a project to discover novel inhibitors of protein tyrosine phosphatase-1B.[114] In this case HTS and VS cannot be directly compared because: (1) different libraries were screened, the HTS one was not very drug like; (2) the assay conditions used for VS hits were more permissive than the ones used in HTS and (3) the VS hits were screened using a medium-throughput assay probably more accurate and sensitive than the HTS. As a result, the authors reported a value of 1700 as an upper estimate of the EF. Finally, a docking-based VS performed at Vernalis to identify Hsp90 inhibitors resulted in a hit rate of 1%, approximately 500-fold greater than HTS.[93] It is interesting to note that in the first case, the EF is in agreement with those reported with pure docking VS experiments using seeded libraries (usually in the 10–50 range[93]), while the other two studies report EFs that are far greater than can be reasonably expected from docking. This can be explained because in the latter cases docking was complemented with a variety of other methods, ranging from drug-like filters to visual inspection of the predicted binding modes and also because they benefited from low- or medium-throughput screening assays, generally more accurate than HTS. This highlights the importance of integrating docking into a wider VS strategy, which will be described below.

3.2.2 *De novo* Design. It has been estimated that more than 10^{60} organic molecules could exist with MW $<$500 Da.[115] Even though the number of molecules needed to cover the drug-like chemical space is bound to be much lower,[116] it is apparent that VS libraries (usually containing 10^5–10^7 chemical structures) cover only a small fraction of the potentially interesting chemical space. As its name suggests, *de novo* methods[117] design ligands from scratch, hence they are not constrained to a pre-defined library and can exploit the whole chemical diversity. Molecules are constructed merging fragments from pre-defined libraries. The number of solutions is kept within reasonable size by building up only those molecules that are predicted to be complementary to the receptor. The degree of complementarity with the receptor is measured with the same or similar scoring functions used for docking.

The chemical structures proposed by *de novo* methods are novel in most cases. Paradoxically, this is a major limitation because synthetic tractability is generally ignored by the ligand construction algorithms and because the predicted binding affinity is very poor. These deficiencies often lead to proposed chemical structures of difficult synthesis and low probability of being active and, ultimately, to waste of valuable synthetic resources. Recent developments in the field have been directed to tackle this issue using two complementary approaches:[118] (a) use *de novo* design program to explore the substitution pattern of known binders and (b) to prioritize the output of the programs by its chemical accessibility. Chemical accessibility has been addressed from different angles, including the use of substructural searches to identify commercially available compounds similar to the *de novo* designed molecules,[119] explicit use of synthetic routes[120] or by limiting the fragment libraries to substructures of drug-like molecules.[121] These recent studies resulted in successful discovery of new chemical series. It is worth noting that *de novo* design methods are highly complementary to fragment screening methods.[122,123] The latter identifies small weak binders that need to grow to become proper leads, the former can greatly benefit from constraining the search to chemical scaffolds experimentally known to bind the active site, because both the limitations of the scoring function and issues around chemical accessibility can be partially overcome.[121] In the light of recent successes and the increasing importance of fragment screening, a revived interest in *de novo* methods is granted.

3.2.3 The Role of Chemoinformatics. All methods of screening, either virtual or real, evaluate lists of molecules and return a subset of this list as hits. Rather obviously, the set of selected molecules is to a large extent pre-determined by the composition of the screening library. Perhaps the most important lesson learned from more than one decade of HTS has been the

Table 6 Chronology of HTS libraries

Period	*Concept*	*Library composition/Filters*
<1990s	Random screening	Historic collections Natural products or extracts
Early 1990s	Combinatorial chemistry	Combinatorial libraries[127]
Early 1990s	Enhanced information content	Molecular diversity[128,129]
Late 1990s	Drug likeness	Rule of 5[54] Solubility and permeability[130]
Late 1990s	Frequent hitters	Reactivity[131,132] Self aggregation[133,134]
Early 2000s	Lead likeness	Lead like filters[135] Ligand efficiency[136]
Early 2000s	Focused libraries	Privileged scaffolds[137,138] Target families and chemogenomics[139,140]

realization that there is a crucial difference between hits (compounds active in the primary assay) and leads (*i.e.* series of molecules that can rapidly evolve into drugs). In consequence, general HTS libraries evolved from being random collections of compounds or mixtures in the early years to drug-like in the late 1990's and continue to evolve to cover a more relevant subset of the chemical space and to increase the chance of finding hits suitable for evolution.[124,125] The evolution of HTS libraries is illustrated in Table 6. Chemoinformatics provides the necessary tools to apply these hard learned experiences (even simultaneously) to library design.[126]

Chemoinformatics also provides tools to analyse and extract information from large volumes of data. Particularly, it is extensively used to generate predictive models, which can then be used to profile compound collections or virtual libraries. Some examples are provided in Table 7.

3.2.4 Integrative VS. When the structure of the pharmacological target is known, structure-based methods can be applied to VS, but this does not preclude the use of any other method. In fact, the best results can be obtained when different tools, be it computational methods or other technologies, are combined together. Industrial VS applications are generally part of a broader strategy to identify new, exploitable leads. This can be best achieved by recognizing the strength and shortcomings of individual technologies, being aware of the specificities of the target, the capabilities of the research organization and designing an *ad hoc* hit identification strategy. We can distinguish two major types of techniques complementary to structure-based methods:

Computational methods. An inspection of recent accounts of successful docking-based VSs reveal that docking is almost invariably supplemented with empirical information,[93,111] this includes the so-called guided docking approaches[145,146] as well as substructural searches,[147] drug-like filters or *in silico* Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) profiling.[144]

Experimental screening methods. HTS and fragment screening methods are highly complementary with VS and should be interconnected rather than perceived as competing technologies.[148] Table 8 provides several examples of how information generated with experimental methods can be used to refine the VS protocol. Ultimately, iterative cycles of virtual and real screenings can be envisaged to identify and evolve those chemical series with greatest potential in a minimum amount of time and cost.

Table 7 Non-exhaustive list of chemoinformatics models for library profiling

Class	*Property*
Solubility	Aqueous solubility Organic solubility
Permeability and distribution	Partition coefficient (Log*P*) Passive absorption (caco-2) Blood-brain barrier Volume of distribution Plasma protein binding P-glycoprotein (PGP) substrate
Metabolism	Cytochrome P450 (CYP) inhibition: CYP2D6 CYP3A4
Toxicity	HERG Binding
Compound quality or relevance	Drug-likeness Target specificity[141] Hit probability[142]

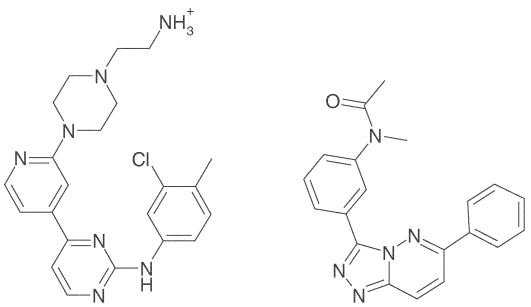
Note: For specific examples see recent reviews by Davis and Riley[143] and Oprea and Matter.[144]

3.2.5 Template or Scaffold Hopping. One classic way of identifying new hits is by redesign of compounds with known activity. This can be limited to modifications of the substituents or a more fundamental change in the core of the molecule. Reasons to modify the basic scaffold of an existing compound include breaking away from a crowded intellectual property (IP) area, removing intrinsic liabilities of the original chemical scaffold or simply to have access to a new chemotype, which may provide some advantage in terms of chemical tractability, potency or overall profile as a drug candidate. Understandably, most examples of scaffold hopping make use of ligand-based methods such as pharmacophore[153] or similarity searches.[154] Nevertheless, these methods are absolutely complementary to structure-based strategies. This has recently been demonstrated by Rush *et al.*[155] in their search for a new molecule capable of disrupting the ZipA–FtsZ complex. A hit had previously been identified by HTS (molecule **1** in Figure 2), but the scaffold presented toxicity and patentability issues. Their VS method of choice was a shape-based molecular similarity approach, but the structure of **1** bound to ZipA had been determined and was exploited in two different ways: on the one hand, it was not necessary to consider several possible conformations of the reference ligand because the bioactive conformation had been experimentally determined and on the other, those initial VS hits presenting overlaps with the volume of the receptor were discarded. After this process, 29 molecules were selected, most of which presented some degree of activity. Most importantly, the new molecules shared the same binding mode as the original HTS

Table 8 Various sources of empirical information and possible uses in VS

Information	*Common sources*	*Possible uses*
Receptor structure	X-ray Homology modelling	Direct use (docking, *de novo*) Extraction of interaction patterns[149] Comparison with other binding pockets[150] Property filters: Size Polarity Charge "Warhead" moiety
Ligands	Published information HTS Fragment screening	Use of ligand-based methods to pre-filter the library: Descriptors Similarity Pharmacophore VS test experiments to optimize docking or *de novo* protocols: Choice of Scoring Function Definition of receptor (*e.g.* water molecules) Learning tools to identify binders
Binding modes	X-ray of protein–ligand complexes	Focused docking (tethered scaffold) Focused *de novo* design (around known scaffolds) Similarity-guided docking[151] Pharmacophore-constrained docking Learning tools to detect incorrect binding modes[152]

hit but provided much better starting points because they did not show the same cytotoxic effects, had less IP concerns and better binding efficiency.[155]



1 HTS hit
K_D = 12 μM
MW = 425.0

2 New inhibitor
K_D = 83.1 μM
MW = 343.4

Fig. 2 Reference compound (**1**) and example of hit (**2**) identified in a scaffold-hopping exercise (Rush et al. 2005)[155]

3.2.6 Target Hopping. In the same way as the structure of a target enables us to identify compounds that bind to it, taking the reverse approach, new binding sites can be found for known drugs. Remarkably successful applications of inverse docking have been described,[156,157] suggesting that this strategy could be used to predict secondary targets of a molecule that may elicit toxicity, secondary effects, increased metabolism, *etc.* In current pharmaceutical research, the concept of target hopping is, nevertheless, mainly associated with chemogenomics.

Chemogenomics aspires at describing the interaction of all possible drugs with all possible targets.[158] To a certain extent, this can be done experimentally,[159] but considering the vast size of both the proteome and the chemical collections, a complete interaction matrix is simply out of reach by any experimental means. A more focused approach is therefore used, where drug-discovery techniques are applied in parallel to several members of a given protein family. Here the aim is to exploit synergies gained from targeting closely related binding sites. Central to this approach is the premise that there is a balance between cross reactivity and selectivity, and the confidence that this balance can be tilted at will in one or the other direction. For example, hit identification is facilitated by searching libraries of compounds designed to be active against a certain protein family (*i.e.*, non-specific compounds). Specificity can be built in at later stages, often by exploiting certain areas of the binding site known to be diverse within the family. A chemogenomics approach does not require structural information but when this is available, as is the case of the large family of protein kinases, a whole new array of methods can be applied.[139,140,160] Just to mention a recent example, Vertex scientists have used the experimental binding mode of frequent kinase hitters to derive a pharmacophore model, which has the ablity to recognize molecules that bind to protein kinases in a non-specific manner.[161]

3.3 Hit to Lead

Once a hit is found, whether it is by experimental or computational means, the next big step in a discovery pipeline is to turn it into a lead. The hit must be optimized to improve both potency and ADMET properties, with the help of *in vitro* and *in vivo* screens. Knowledge of the way the hit interacts with the target is crucial to guide potency improvements and gaining insight on where to modify its scaffold and/or chains in order to modulate several ADMET properties such as solubility, metabolic stability and toxicity. This makes the application of structure-based modelling techniques at this stage of development as important as in hit identification. We will first consider the problem faced in elucidating the binding mode of the hit, Second, once this is known, the strategies and modelling techniques used to increase its biological activity, and finally, review some cases where details of the ligand–receptor complex have allowed the rational modification of the hit in order to improve ADMET properties, making special emphasis on selectivity issues potentially linked to toxicity.

3.3.1 Binding Mode Determination. In a rational approach to drug discovery, after finding a hit, the central question raised is

how it binds to its target. If the hit has been found in an HTS campaign, there are in principle, no structural clues as to how it binds to the receptor. If the structure of the target is known, a combination of *in silico* techniques can be applied. Initially, docking can be used to suggest possible binding modes of the hit in the target's active site. In the absence of a crystal structure of the target, docking can still be a very useful tool if a homology model of the target can be built based on a related template of known structure. Recently, researchers at Astra Zeneca have disclosed a structure-based approach using this strategy.[162] A hit was found in a HTS campaign for IkappaB kinase 2 (IKK2) inhibitors. Because the binding mode was unknown, first a crystal structure of the inhibitor with a distantly related kinase, JNK1 was obtained. It was seen that the inhibitor was an ATP competitive molecule that bound in the ATP binding site of JNK1. This gave clues as to what the binding mode could be in the ATP binding site of IKK2. A homology model of IKK2 was built based on the structures of distantly related kinases, and the hit was docked inside this new structure. Once the binding mode was known, it could be rationalized which chemical groups closely interacted with the different protein residues, which parts of the molecule were pointing towards the interior of the protein and which were solvent exposed.

If the hit has been found with a modelling method such as high-throughput docking, a pharmacophore built from a crystal structure, or other approaches, there may be straightforward clues as to how it is interacting with its receptor. There are numerous instances where active hits selected from high-throughput docking have later been confirmed to bind the way they were predicted by the docking program.[93,110] However, it must be borne in mind that, although in the ideal cases the docking pose can be very close to the actual location, there might be many cases in which the rigidity of the receptor prevents finding some of the right ligand–receptor interactions. In those cases where induced fit is of relevance, refinement of the docked pose by more computer intensive techniques such as MD or MC, can alleviate some of the distorted interactions and give a more realistic model. One of such cases has recently been published on the binding mode of hydantoin-based antagonists of lymphocyte-associated antigen-1 (LFA-1).[163] First the antagonists were docked to the I-domain of LFA-1 with restraints derived from a combination of experimental techniques such as antibody mapping and photoaffinity labelling. The proposed binding mode was then refined by a series of explicit solvent MD runs followed by minimization. The final modelled structure was afterwards seen to have only 0.64 Å RMSD from the experimentally derived one.[163]

Irrespective of the source and technique used in hit finding, sometimes multiple reasonable binding modes are possible. In those cases where a SAR around the hit is available, the changes in activity can give clues on what is the correct binding mode among those detected by docking. Nevertheless, there is frequently no SAR available at the beginning of the hit to lead phase. In such cases, it is reasonable to use some of the *in silico* techniques routinely used for improving the potency of a hit such as linear response (LR) or MM-PBSA, (see a full description below). Because these techniques more reliably predict the ΔG of binding than the prototypical scoring functions used for docking, they can more successfully identify the correct binding mode of a compound as opposed to incorrect ("decoy") poses generated by a docking program. An illustration of this strategy was reported for the elucidation of the binding mode of efavirenz to HIV-1 RT.[164] Efavirenz was first docked to the crystal structure of HIV-1 RT with the Dock program, and five possible binding modes were found. Next, MM-PBSA calculations were carried out on a 500 ps MD trajectory to the five possible binding modes. One of them was clearly pointed out by these calculations as the more stable, having a binding free energy of 7 kcal/mol more favourable than the second pose. Subsequent cocrystallization of the HIV-1 RT-efavirenz complex confirmed the correctness of the modelled complex.

3.3.2 Improving the Potency of the Hit. The affinity of the hit for its target can be increased by modulation of either or both the enthalpy and the entropy associated to complex formation. Lowering the loss of entropy upon binding can be achieved both by burial of polar groups in a hydrophobic environment of the receptor, the so-called hydrophobic effect, or by eliminating loss of degrees of freedom of the small molecule upon binding *via* rigidification.[2,3] The enthalpy is usually increased by optimizing the steric and electrostatic fit of the complex, increasing the strength of the van der Waals and electrostatic interactions or gaining additional ones. The formalism needed at this stage is similar to some described above for hit finding. In order to apply a rational approach, one must have a way of evaluating the free energy of complex formation or its changes. Thus, a typical docking engine based on a scoring function should in principle be enough to guide the optimization. However, as has been explained above, one of the most important limitations of docking is the rigidity of the receptor. This limitation, among others, severely restricts the accuracy of this technique, which currently can at best be used to discriminate between mili-, micro- and nanomolar binding affinities.[165] Because the goal at this stage is to fine-tune the activity, better accuracy is needed. Fortunately, research is done exclusively on the hit and its derivatives, making the number of molecules to handle computationally much smaller than in hit finding. This allows the application of more thorough ways of sampling, better exploring the configurational space of the ligand–receptor complex, which also has an impact on the accuracy of the techniques.

Once the 3D structure of the receptor and the binding mode of a hit are known, one of the simplest techniques that can be used as a guide to improve the potency is a molecular interaction energy analysis of the target's active site (described above for active site detection). Typically, the hit is removed, only the active site is immersed into a cubic grid, and the interaction potentials between receptor and the probes are calculated. This can be subsequently translated into new chemical modifications to the hit being optimized, which are consistent with the interaction energy analysis. Although this technique is still in use, the major drawback of this type of calculations is their qualitative nature, which does not allow to use them as a prioritizing tool.

Among the low-throughput techniques available for hit optimization and quantitative rank ordering of small (*ca.*

10–100 compounds) sets, one of the less computationally demanding ones is the COMparative BINding Energy (COMBINE) method.[166] This method is similar to the classical QSAR methodology. QSAR techniques correlate binding affinities with a set of physicochemical descriptors of the ligands. Similarly, in COMBINE the free energy of binding is expressed as a linear combination of different weighted terms. However, the terms are individual residue-based ligand–receptor interaction energies (electrostatic and van der Waals) computed by MM minimizations of the set of molecules under study in the active site of the receptor. The interaction energies are first subjected to a chemometrical analysis devised to separate the mechanistically important ones from the background noise. Because only MM minimizations are required, the sampling achieved is very crude, but translates into a higher throughput capacity of the method. COMBINE was first tested on a set of 26 human synovial fluid phospholipase A2 inhibitors,[166] with an encouraging predictive ability. The method has later been refined and adapted to incorporate desolvation effects, which were shown to increase the predictive ability of the linear regression model in a set of HIV-1 protease inhibitors.[167] A further application was reported on the use of COMBINE for the derivation of a predictive regression model for influenza neuraminidase type A inhibitors[168] in which the role played by a particular crystallographic water molecule was found to improve the model. Very recently, the use of this technique has been coupled to a docking engine and found to improve the recognition of actives vs. inactives as compared to only docking energies for a series of factor Xa inhibitors.[169] While COMBINE represents a step beyond docking in terms of sampling and accuracy of rank-order prediction, this method still has several limitations. Radically different binding modes by different scaffolds may involve different residue interaction patterns, which could lead to inaccurate predictions. Because the entropic term is not accounted for, only series of molecules with very similar flexibility can be properly addressed. Also, the minimization procedure applied for relaxing the different complexes might not be sufficient to find the correct interactions. Finally, because it is a parametric method, a set of known experimental binding activities must be available for a training set of molecules before the model can be built, which limits its applicability.

The linear interaction energy (LIE) or LR first introduced by Aqvist *et. al.*[170] represents a step further in the modelling of ligand–receptor complexes. Although it is also a regression method where the absolute free energy of association is calculated by a sum of different weighted contributions, it is more thorough than COMBINE because it includes solvent explicitly and performs ensemble averaging derived from extensive sampling of configurational space. The method requires two simulations per inhibitor, one freely in solution and the second on the solvated inhibitor–receptor complex. This allows calculation of the van der Waals and electrostatic interaction energy of the ligand with its environment in both states, bound and unbound. The free energy of binding is then expressed as a linear combination of these two energy differences. The two differences must first be calibrated with a training set in order to derive the weighting terms and build a predictive model. The extensive sampling can be achieved by both MC or MD simulations. Although originally the method only accounted for differences in van der Waals and electrostatic interactions, it was later expanded to account for other terms such as changes in SASA. Additional descriptors such as changes in the number of hydrogen bonds, internal energy of the ligand or the number of rotatable bonds were added to the original LR approximation in subsequent versions of the method,[171–173] effectively making it an ensemble averaged empirical scoring function analogous to the ones used in docking. Thus, in a study of 20 thrombin inhibitors, regression models were built based on 3–5 descriptors that reproduced experimental binding affinities with an r^2 of 0.7–0.8 and rms errors of 1–1.3 kcal/mol, an accuracy suitable for hit optimization.[171] The terms found most important for the prediction of binding affinities were the internal energy change and loss of hydrogen bonds for the ligand upon binding, enhancement of van der Waals contacts with the protein and number of rotatable bonds. However, in another study conducted on a set of 40 HIV reverse transcriptase inhibitors, the most important terms were not identical, being of special importance the removal of exposed hydrophobic surface area upon binding, the so-called hydrophobic effect.[172] This illustrates the difficulty in finding a universal LR expression that can be used to predict the affinity of any organic molecule for any biological receptor.

Changes in the microenvironment of the targets and the actual nature of the ligand–receptor interactions translate into sensible variations of the different terms. In this respect, a very recent communication analysed the feasibility of obtaining a universal LR equation for the prediction of affinities for the kinase family.[174] Three different kinase systems, lck, p38 and cdk2 were chosen. As the first step, regression models were built for each one of them separately by using three training sets of inhibitors, one for each target, with reasonably good correlation coefficients in the 0.7–0.8 range. The terms found to be most significant were changes in van der Waals and Coulombic ligand–receptor interactions and changes in hydrogen bonds upon binding for lck and cdk2 (see expression 1 and 2).

$$\text{cdk2}: \Delta G_{\text{calc}} = 0.1\langle \text{EXX}-\text{C}\rangle + 0.11\langle \text{EXX}-\text{LJ}\rangle - 0.216\langle \Delta \text{HB}_{\text{total}}\rangle - 0.135$$

$$\text{lck}: \Delta G_{\text{calc}} = 0.0989\langle \text{EXX}-\text{C}\rangle + 0.257\langle \text{EXX}-\text{LJ}\rangle - 0.32\langle \Delta \text{HB}_{\text{total}}\rangle + 0.623$$

$$\text{p38}: \Delta G_{\text{calc}} = 0.0644\langle \text{EXX}\rangle + 0.00691\langle \Delta \text{FOSA}\rangle - 0.76\langle \text{QP}\log P_{o/w}\rangle - 0.623$$

However p38 (expression (3)) the terms found were Coulombic interaction energy changes, together with changes in exposed hydrophobic surface area upon binding and the octanol/water partition coefficient for the ligands, therefore making it a completely different expression from the ones derived for the other two kinases. As in many other LR studies, the coefficients of the different terms make sense from a physical viewpoint. The signs for EXX-C (ligand-enzyme Coulombic interaction energy), EXX-LJ (ligand-enzyme Lennard-Jones

energy) and EXX (the sum of EXX-C and EXX-LJ) are positive, implying that a good electrostatic and steric fit lowers the ΔG of binding (bound–unbound inhibitor). The sign for ΔHB_{total} must be negative to reflect the penalty for hydrogen bond loss upon binding. In expression (3), reduction of exposed hydrophobic surface area upon binding (ΔFOSA) and increased hydrophobicity are also favourable and so have a positive value. The leave-one-out predictive correlation q^2 for each of the three expressions was found to be 0.72, 0.68 and 0.60 respectively, enough to be used in a hit optimisation stage.

As a second step, combinations of two kinase datasets were used to derive an LR equation that was later used to predict the activities of the third kinase. The correlation coefficients in these three cases between predicted and experimental ΔGs were 0.53, 0.7 and 0.7. Although the three models shared the same types of descriptors, the coefficients multiplying them varied. As a final step, the three kinase datasets were combined (totalling 148 compounds) to obtain a single "universal" LR equation for the kinases (see expression (4)).

$$\Delta G_{calc} = 0.0848\langle EXX\rangle - 0.293\langle \Delta HB_{total}\rangle + 0.0123\langle \Delta SASA\rangle - 3.11(L_{corr}) + 3.08$$

This equation had a correlation coefficient, r^2 of 0.69, an rms error of 0.77 kcal/mol, and a leave-one-out q^2 of 0.66, again featuring changes in the Coulombic and Lennard–Jones interaction energies, loss of hydrogen bonds and changes in exposed hydrophobic surface area upon binding as most important terms.[174] (the term named $L_{corr}$was introduced in the study as an indicator variable, with value of 1 for an lck inhibitor and 0 otherwise). Although the reliability of these models calls for improvement, it shows this tool is accurate enough for guiding synthetic efforts even within protein families.

The biggest alternative to the LR methodology is the MM-PB/GB(SA) approximation developed by the Kollman group.[52] In this new method one predicts the free energy based on an MD simulation in explicit solvent and counterions. The dehydrated trajectory is later postprocessed, and the free energy is calculated as a sum of the molecular mechanical term (which accounts for bond, angle, torsion, van der Waals and electrostatic terms), a solvation free energy term calculated with the PB or the GB method plus a surface area term, and a final term that accounts for entropy changes estimated by quasiharmonic or normal-mode analysis. The biggest advantage of the method with respect to LR is that it is non-parametric, and thus one does not need to utilize a set of structures and experimental activities to train the model. Furthermore, the method can be applied for estimating the free energy of ligand–receptor association based solely on a single MD run, that of the complex, instead of 2 as in the LR method.[52] One of its first applications was the study of a set of avidin inhibitors. In this study, a remarkable correlation r^2=0.92 was achieved. Furthermore, it was stated that the method could yield better results than LR, since the latter gave an r^2=0.55 for the same set of molecules.[175] In a recent application of the method, calculated and experimental binding free energies were compared for a set of cathepsin D inhibitors generated by a combinatorial library approach.[176] Although the free energies of association calculated with a standard docking tool such as Dock were found not to correlate with the experimental values, the MM-PB(SA) estimated affinities gave impressive results, with an r^2=0.98 and an average error of approximately 1 kcal/mol, enough to be used as a prioritizing tool.

A very recent application of the MM-PB(SA) approach has been disclosed in which this technique is used in combination with experimental techniques for the development of high affinity phosphodiesterase inhibitors.[177] First, a fragment-based high-throughput X-ray crystallography screening was performed in order to find low molecular weight inhibitors with only marginal *in vitro* activities. At this stage of scaffold discovery, from a library of 20,000 compounds, 316 were found to give more than 30% inhibition at 200 μM. From this pool, 107 were cocrystalized with the target PDE4. One of the interesting scaffolds was found to be a pyrazolo derivative (PCEE) with an IC_{50} of 82 μM and only 168 Da molecular weight. In a second step, this newly found scaffold was tested to see whether its binding mode was consistent with chemical optimization. In order to make sure that the binding mode of the core would be maintained, a small series of derivatives were synthesized and cocrystallized in a second round based on the information derived from the first round of crystallography. From this pool of new derivatives, a phenyl-substituted derivative (PhPCEE, 244 Da. molecular weight) was chosen because of its improved activity, with an $IC_{50}$of 270 nM. In an effort to further increase its activity, from PhPCEE and the available reagents, more than 100 compounds were designed *in silico*. These molecules were docked to the crystal structure of PDE4 bound to PhPCEE and their ΔG of binding was predicted with the MM-PB(SA) approach. Out of this group, 10 compounds were synthesized and their IC_{50} values were obtained. The correlation found between predicted and actual values was 0.92, confirming the good reliability of the technique. The most active compound tested showed an IC_{50} of 21 nM, that is, a 4000-fold increase with respect to the starting structure.[177]

The only structure-based modelling techniques considered more reliable than the ones mentioned above are those based on statistical mechanics.[1,52] FEP and TI represent the most rigorous approach to simulating free energy changes, and should therefore be also applied in the drug-discovery process. However, these methods have two severe limitations, great computational expense and problems with convergence, which limits its application to small structural changes between the two inhibitors to be compared. For these reasons, they are considered impractical and very seldom used in the drug-discovery context. To illustrate the reliability of this type of calculations a study was published in which the changes in potency of a congeneric series of p38 inhibitors were calculated with scoring functions and also with the more rigorous TI calculations.[165] The results of this publication clearly pointed to the complete unreliability of the high-throughput techniques for the prediction of small changes in free energy of binding, and how the latter can be effectively used in a hit-optimization context. Because of its precision, this technique has recently been applied in a considerable number of other situations, such as the study of the effect of protein mutations on complex formation (for instance, see the study of HIV-RT mutations and how they affect the binding of several drugs[178]), the

change in orientation of lateral chains in an active site depending on the actual electronic structure of the inhibitor (*e.g.* see the study of COX2 complexed with rofecoxib and celecoxib and other diaryl-heterocyclic inhibitors[179]), for the validation of a proposed binding mode (see *e.g.* the binding of tacrine-huprine hybrids to acetylcholinestarase[180]), and many others.

Finally, there exist other less thoroughly tested methods to be used in the hit to lead process. One of such methods has recently been proposed as an alternative to LR and MM-PBSA.[181] In this method, the free energy of binding is calculated from descriptors obtained from a MD trajectory performed *in vacuo*. The binding free energy is expressed as a sum of weighted terms, just as in LR. The terms are an electrostatic interaction energy, a term accounting for the buried surface upon complexation, and a solvation term which is calculated by postprocessing the MD trajectory and solving the PB equation at selected snapshots. The advantage of the method with respect to MM-PBSA is that the MD simulation is performed *in vacuo*, in contrast to the former, which is carried out in explicit solvent. Also, it does not need an MD trajectory of the ligand alone, as in LR. This method has only been tested on a training series of HIV protease inhibitors, and a remarkable r^2 of 0.91 was obtained. However, on being used for the prediction of a 25-member test set the correlation coefficient dropped to 0.64, although it must be borne in mind that experimental free energies of binding only spanned 3 orders of magnitude. The novelty of this method together with the lack of additional applications precludes its comparison with other methods to be used in the hit to lead stage.

3.3.3 Modulation of ADMET Properties. A deep insight on how to change the chemistry to alter ADMET related parameters can be gained by simply knowing the binding mode of the candidate. Thus, for instance, the development of p38 kinase inhibitors has recently met problems because of the hydrophobic character of its particular ATP binding site. This has translated into problems not only of solubility but also of P450 inhibition.[182,183] Several groups have coupled experimental and *in silico* techniques for the determination of the binding mode, which has been exploited to functionalize the different scaffolds with solubilizing groups exposed to solvent. The case of the pyridinyl-heterocycle family of inhibitors has been known to be problematic due to CYP 3A4 inhibition.[183] Once their binding mode has been determined, the pyridine that hydrogen bonds to the hinge region, which is also responsible for CYP inhibition, has been replaced to pyrimidine lowering the affinity of this class of molecules for the cytochrome.

Although structure-based modelling techniques can give clues that directly impact ADME optimization, toxicity in particular deserves a special attention. Many of today's interesting targets with known 3D structure belong to large protein families such as kinases, nuclear receptors and proteases. These families have from a few dozens to several hundred members in the human genome (see for example an analysis of the human kinome[184]). The degree of structural homology within these families is high. Usually the active sites of many family members are nearly identical in terms of electrostatics and shape, making the development of potent and selective inhibitors a challenging task. Usually, a lack of selectivity for these targets translates into toxicity problems. This has fuelled the rise of chemogenomics approaches to drug discovery,[158] whereby biological activities of series of compounds are studied on a family wide-basis instead of on a single target. Very interesting *in silico* applications have recently been published for the kinase family. One of them[150] attempted to classify a group of kinases using 3D molecular interaction potentials derived from their structure. A second one classified this family of enzymes on the basis of small molecule selectivity.[160] A third analysis shows how chemogenomics can be used for finding or avoiding dual inhibition of a pair of related enzymes.[140] Undoubtedly progress in the structural determination of many of the target family members will spur the development of more complete and reliable *in silico* chemogenomics studies, which will have a positive impact on toxicity liabilities due to the rational fine tuning of selectivity within protein families.

4 Conclusion

There are continuous methodological developments in computational chemistry which, coupled with an ever increasing availability of computational power, are rapidly advancing the capacity of the field to make an impact not only in the drug discovery arena but also in the broader field of molecular biology. Our tools remain, nevertheless, imperfect and inaccurate. This is particularly true for binding and conformational free energies when a macromolecule is involved. But the good news is that molecular modelling techniques have reached a stage where they are definitely useful. An inspection of high-impact medicinal chemistry journals shows that molecular modelling is an integral part of the drug-discovery process in most research organizations and, most importantly, the combination of computational chemistry with detailed knowledge of the structure of pharmacological targets has been particularly successful, and a number of drugs reaching the market have, in fact, resulted from this fertile interaction.[185] As the knowledge about biological systems continues growing at gigantic pace, molecular modelling is destined to assume an ever more central role in the integration of information of genetic, structural, biological pathways or chemical origin and, with it, increasing responsibility to deliver effective medicines and to make possible the anxiously awaited increase of productivity in the pharmaceutical industry.

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