CHAPTER 2

The Changing Landscape in Drug Discovery

HUGO KUBINYI

D-67256 Weisenheim am Sand, Germany

2.1 Introduction

Looking back on 40 years of research in medicinal chemistry, later in molecular modeling and combinatorial chemistry, I see a significant change in the science and art of drug discovery. When I started my industrial career in 1966, in the search for new drugs, my colleagues and I synthesized a few compounds per week, at most. The biggest problem was to convince the pharmacologists to investigate them in due time, because they were still busy with the compounds delivered the weeks before. The chemical structures of our candidates resulted from working hypotheses that were often based on poor evidence. Once the compounds showed some activity in animals, we hoped that this would also be the case in humans. This situation, as well as books on drug discovery from that time, like Frank Clarke's *How modern medicines are discovered*,[1] Alfred Burger's *A Guide to the Chemical Basis of Drug Design*,[2] and Walter Sneader's *Drug Discovery: The Evolution of Modern Medicines*,[3] sound like stories from an ancient time, long ago. Nevertheless, they tell us how medicinal chemistry, biology, and pharmacology successfully(!) worked together to discover new medicines.

In an excellent review, Ralph Hirschmann characterized the decades between 1950 and 1990 as "Medicinal chemistry in the golden age of biology".[4] Indeed, this time was the golden age of drug discovery. New research results in biochemistry and biology paved the way from neurotransmitters and hormones to more-or-less selective agonists and antagonists of G protein-coupled receptors (GPCRs) and nuclear receptors. Within this relatively short period, Paul Janssen and his company were able to introduce about 80 new drugs into human therapy, many of which are still highly valuable therapeutics.[5] Whereas this unique yield may be (correctly) interpreted as the success of a genius, one should also consider that his research techniques were at the frontier of science. He used a toolbox of biologically interesting substructures (today we would call

them "privileged fragments") and assembled them in different combinations, long before combinatorial chemistry or fragment-based design appeared on the scene. Nonlinear mapping methods were applied to investigate the relationships between animal and *in vitro* test models, and to characterize the biological activity profiles of the compounds.[6] But the main ingredient for success, as in most other companies, was a deep understanding of the underlying structure–activity relationships (SARs) and creative intuition in medicinal chemistry.

Nowadays genomics, proteomics, combinatorial chemistry, high-throughput screening (HTS), structure-based and computer-aided design, and virtual screening have completely changed the strategies of the drug discovery process. Without focussing on experimental techniques, the most important computational approaches in drug discovery are discussed in the following sections, which evaluate their strengths and limitations.

2.2 QSAR – Understanding Without Prediction

The very first computer-aided approach in drug design developed in the early 1960s, when Corwin Hansch started the QSAR (quantitative structure–activity relationships) discipline.[7,8] He considered drug action to result from two independent processes, that is:

(i) transport of the drug from the site of application to the site of action, and
(ii) non-covalent interactions of the drug with its binding site at a receptor.

As neither very polar nor very lipophilic compounds have a good chance to permeate several lipid and aqueous phases, he formulated a nonlinear lipophilicity relationship for the transport. Then, following a proposal by his postdoc Toshio Fujita, he combined lipophilicity terms and electronic parameters, and later molar refractivity and steric terms also, in a linear free-energy related (LFER) model to describe the ligand–receptor interaction. His third contribution was the definition of a lipophilicity parameter π, in the same manner as Louis Hammett had defined the electronic σ parameter 30 years before. Whereas Hansch and his group, as well as many others, were able to derive thousands of QSAR models for all kinds of biological activities, this approach was not much accepted by medicinal chemists. The very same happened to 3D QSAR methods, like comparative molecular field analysis (CoMFA), which were introduced about 20 years later.[9–12]

In principle, 3D QSAR is more powerful than classic QSAR, because:

(i) 3D structures are considered instead of only 2D structures;
(ii) more heterogeneous sets of compounds can be included than in classic QSAR;
(iii) molecular fields are calculated instead of just substituent constants;
(iv) contour maps show the effect of certain properties in certain regions.

However, these advantages are associated with several problems:

(i) most often neither the bioactive conformation nor the binding mode of the molecules of the data set are known;
(ii) a model-based superposition of the molecules always remains hypothetical;
(iii) minor displacements of the box around the molecules generate different and irreproducible results, because of the artificial cut-offs of the Lennard-Jones and Coulomb potentials – this problem can be avoided in the CoMSIA (comparative molecular similarity index analysis) modification[13];
(iv) variable selection produces fragmented contour maps that are difficult to interpret – instead of single variables, regions should be selected.

Thus, two reasons may be responsible for the lack of acceptance of QSAR and 3D QSAR by medicinal chemists: first, a detailed knowledge of statistics and much practical experience are needed to apply these methods in a proper manner and, second, even ''good'' models, with sufficient internal predictivity, are often poor in test-set prediction.[14–17] In particular, the best-fitting QSAR models, which result from variable selection and are validated by all reasonable statistical criteria, including cross-validation and y scrambling, are externally less predictive than models with inferior fit, an observation that has been called the ''Kubinyi paradox''.[18,19] However, it's no paradox, but results from the fact that these best-fitting models include variables that fit the error in the data, whereas some other models do not. The variation in test-set prediction results from the distribution of data with major experimental error (not necessarily outliers): if they are included in the test set, external predictivity is poor; if they are included in the training set, fit is poor, but external predictivity may be much better.[17]

So, what remains from QSAR for the medicinal chemist? The best answer has been given by Robin Ganellin, one of the leading medicinal chemists of our time, when he was asked by Steve Carney, ''Has there been a single development that, in your opinion, has moved the field of medicinal chemistry ahead more than any other?'' and Ganellin responded, ''I would go back to the 1960s to the work of Corwin Hansch on the importance of lipophilicity. . . . I think that changed the way of thinking in medicinal chemistry . . . . I think that the application of physical organic chemical approaches to structure–activity analysis have been very important''.[20] There is nothing more to say. Today, ligand–receptor interactions are considered in terms of hydrophobic interactions, polarizability, and ionic and neutral hydrogen bonds. The influence of lipophilicity, as well as of the dissociation and ionization of acids and bases, on transport and distribution is well understood. Medicinal chemists, who did not care about the pK_a values of their acids or bases, are now well aware of the risks that arise from those values being too far away from 7, the neutral pH value.

2.3 Gene Technology – from Mice to Humans

Gene technology created a lot of hype and it still creates a lot of fear. However, many of the anticipated benefits have not resulted. In a critical analysis, Glassman presents a long list of technologies for which the "initial hype of healthcare innovations did not live up to expectations",[21] including immunotherapy for cancer, stem cell technology, antisense technology, pharmacogenomics, genomics-based target identification, and gene therapy. He argues that the targets of several blockbuster drugs [statins, proton-pump inhibitors, leukotriene antagonists, selective serotonin reuptake inhibitors (SSRIs), taxol, angiotensin-converting enzyme (ACE) inhibitors, antihistamines, and kinase inhibitors] would not have been discovered by a systematic search for mutated "disease" genes. However, he admits that these targets might have been discovered through knockout technology.

Gene therapy has not yet been a success and we do not know whether it will be applicable in the future. Also, genomics-based target identification has not delivered to the expected extent. On the other hand, gene technology contributes to the production of human proteins for substitution therapy [insulin, growth hormone, erythropoietin (EPO), *etc.*] and to a better understanding of the function of enzymes and receptors. By far, the most important application of gene technology is in drug research. After the identification of a potential target, by any technology, the deoxyribonucleic acid (DNA) or messenger RNA (m-RNA) sequence of the corresponding gene directly provides the protein sequence. In many cases, even the folding and the function of a protein can be derived from its sequence. Larger amounts of the protein are produced in bacteria, insect cells, or higher organism cells, which enables the development of (high-throughput) screening models and, if more material becomes available, also a 3D structure elucidation by protein crystallography or multidimensional nuclear magnetic resonance (NMR) methods. Going back 25 years, this was the situation: with certain exceptions, we could only test in animals or with organs or other material from animals, which too often produced misleading results. Now we screen and develop our potential drug candidates with human (!) proteins – this is most probably the biggest achievement of gene technology for the benefit of mankind.

Before the sequence of the human genome became known, there were estimates of about 100 000 or even more human genes. This number had immediately to be corrected to about 30 000–35 000; recent estimates are closer to 20 000–25 000 than to these larger numbers. In the year 2000, Jürgen Drews counted 483 targets of current therapies and he speculated that, in total, there might be about 5000–10 000 drug targets, starting from an estimate of about 1000 "disease" genes and 5–10 proteins linked to such a disease gene.[22] Hopkins and Groom arrived at much smaller numbers in their estimate of the "druggable genome". Starting from a number of 30 000 genes in the human genome, they assumed that about 10% are disease-modifying genes and about 10% are druggable genes (*i.e.* genes for which the corresponding proteins can be modulated by a small molecule). As both subsets do not completely overlap,

600–1500 drug targets were estimated.[23] This estimate has to be criticized, because the term ''druggable genome'' is highly misleading. The relatively small number of genes within our genome codes for a few hundred thousands different proteins, because of alternative splicing and post-translational modifications. Many more potential drug targets result from protein-complex formation – a few protein chains form a multitude of different receptors and ion channels [*e.g.* the integrins, heterodimeric GPCRs and nuclear receptors, γ-aminobutyric acid (GABA) and nicotinic acetylcholine receptors, *etc.*].[24] Without speculating about numbers of druggable, disease-relevant proteins, we can conclude that there are many more potential targets than anticipated by Hopkins and Groom; the number might even surpass Drews' estimate. Indeed, the situation is more complex – several drugs, especially central nervous system (CNS) drugs, do not act against only one target, they modulate several targets at the same time.

Thus, two questions are associated with target-based screening. The first is whether high target selectivity is a desirable or unfavorable property? In certain cases it might be imperative to have such a high target selectivity [*e.g.* for an human immunodeficiency virus (HIV) protease inhibitor], but even here activity against a multitude of protease mutants of resistant strains is highly desirable. In other cases, it might be good to have a defined but broader selectivity against several related targets [*e.g.* metalloprotease inhibitors, kinase inhibitors (newer investigations show that even so-called ''selective'' inhibitors, like imatinib, show a broader spectrum of inhibitory activities against several kinases[25] than originally anticipated), and especially CNS-active drugs, *e.g.* the atypical neuroleptic olanzapine, which has nanomolar affinities at more than a dozen different GPCRs and the 5-hydroxytryptamine ($5HT_3$) ion channel]. Nobody will ever know whether this promiscuous binding behavior shows the right pattern or whether activities at a certain receptor should be higher or lower; but even if we knew, how could we design a compound that has exactly this slightly modified binding pattern?

The second question is, ''Do we lose too many potential drugs by target-based screening?'' The unexpected prodrug sulfamidochrysoidine would not have been discovered in cell culture; omeprazole acts only in acid-producing cells, after acid-catalyzed rearrangement; aciclovir is monophosphorylated only by a viral thymidine kinase, thus it works only in virus-infected cells. For this purpose, chemical biology, which aims to discover new leads by searching for phenotypical changes in cells or small animals, is a step in the right direction.

2.4 Combinatorial Library Design – Driven by Medicinal Chemistry

Combinatorial chemistry really had a poor start. In a bid for numbers, huge libraries were prepared as more-or-less undefined mixtures of compounds, driven by chemical accessibility. Biological activities, if discovered, often disappeared after deconvolution (*i.e.* the preparation of pure, single compounds

that should be present in the mixture). Even when chemists realized some of the problems of such libraries, they searched for potential solutions in the wrong direction. In the 1990s, similarity and diversity were a big issue in chemoinformatics, despite the fact that the similarity of compounds can only be defined from a chemical point of view, never from a biological perspective.[26] But even from a chemical point of view, how similar or dissimilar are benzene and cyclohexane? Looking at aromaticity – no similarity at all; looking at lipophilicity and many other properties – very similar.

In 1998, Stuart Schreiber impressed organic chemists with the stereoselective synthesis of a library of 2.18 million ''natural product-like compounds'', starting from shikimic acid via a tricyclic intermediate, which allowed specific chemical reactions in many different directions.[27] In a misunderstanding of chemical diversity and in an over-optimistic consideration of its potential for producing biologically active compounds, this approach was called ''diversity-oriented organic synthesis'' (DOS).[28] In a later retrospective, Schreiber had to admit that ''the field of DOS has not yet come close to reaching its goals . . . even a qualitative analysis of the members . . . reveals that they are disappointingly similar. Of even greater concern is that the selection of compounds has so far been guided only by the organic chemist's knowledge of candidate reactions, creativity in planning DOS pathways, and intuition about the properties likely to yield effective modulators. Retrospective analyses of these compounds show that they tend to cluster in discrete regions of multidimensional descriptor space. Although algorithms exist to identify subsets of actual or virtual compounds that best distribute in chemical space in a defined way . . . these are of little value to the planning of DOS''.[29] This is exactly the dilemma of chemistry-driven combinatorial chemistry.

Recently Lipinski and Hopkins presented a cartoon which shows the chemical space as a box with embedded regions that stand for bioavailable compounds [the absorption, distribution, metabolism, and excretion (ADME) space], GPCR ligands, kinase inhibitors, protease inhibitors (in this cartoon they do not overlap with the ADME region – a sad experience in the search for bioavailable thrombin inhibitors).[30] If one understands chemical space as being of almost infinite size and the ''bioactivity regions'' just as very tiny spots, like the stars within our universe, we can more easily understand and accept the almost complete failure of chemistry-driven combinatorial libraries with respect to new biologically active compounds. The situation is even worse – biological activity space seems not to be evenly distributed in chemical space. There are groups of islands with higher density, so-called ''privileged structures'',[31] which definitely have a higher chance to produce biologically active molecules than others. Such privileged structures (*e.g.* the benzodiazepines, steroids, phenethylamines, diphenylmethanes, diphenylamines, and tricyclics, to mention only a few) are also called chemical masterkeys,[32] following the ''lock and key'' principle of Emil Fischer. Searching for ''new'' chemistry increases the risk of ending up with biologically inactive molecules because it avoids the privileged ''activity islands''.

Camille Wermuth, another great medicinal chemist of our time, proposed the "selective optimization of side activities" (SOSA) approach,[33,34] which starts from any side activity of a certain drug and aims to optimize this activity and to generate a new selectivity in this direction. There are several examples from the past where such side activities have been discovered and used to create new drugs, *e.g.* the development of diuretic and antidiabetic sulfonamides from the antibacterial sulfonamides (reviewed by Wermuth[33,34] and Kubinyi[35]).

In 1999, Roger Lahana argued that not a single new lead resulted from HTS and combinatorial chemistry.[36] Whereas this statement was and is wrong, his other conclusion, "when trying to find a needle in a haystack, the best strategy might not be to increase the size of the haystack" is absolutely correct. Another truism was formulated by Ashton and Moloney,[37] "Combinatorial chemistry has certainly failed to meet early expectations. Does this mean the technology has failed? Or does the problem lie in the manner in which the technology has been applied?"

In 1997 Chris Lipinski had already observed that some properties of the Pfizer in-house compounds, especially molecular weight and lipophilicity, developed in a wrong direction. As a consequence of increasing molecular weight and increasing lipophilicity, many screening hits could not be profiled as potential leads. Investigating a collection of drugs and drug candidates, he realized that only a minor percentage of these compounds had a molecular weight >500, a lipophilicity (expressed as log P) >5, more than five hydrogen bond donors, and more than 10 N + O atoms (as a rough estimate of the number of hydrogen bond acceptors). From this observation he defined his now famous "Rule of Five" (also called the Lipinski rule of 5 and Pfizer rule of 5) that low permeability of a molecule is to be expected if more than one of the following rules is violated: molecular weight <500, log $P<5$, no more than five hydrogen bond donors, and no more than 10 hydrogen bond acceptors.[38] Originally intended only as a warning flag for the Pfizer chemists, the rule was immediately accepted by the scientific community. It helped to clear screening collections from inappropriate compounds and to avoid the synthesis of meaningless combinatorial libraries. Nowadays, application of the Lipinski rule is mandatory in compound acquisition and in almost every library design.

Sometimes the Lipinski rule is misunderstood in the sense that it could define a drug-like character of the compounds. This is not the case – it defines drug-like properties with respect to bioavailability, but not drug-like structures; most of the Available Chemicals Directory (ACD) compounds pass the Lipinski filter, but they are by no means drug-like with respect to their structures. In a rare coincidence, two groups at Vertex and BASF independently developed almost identical neural net filters to characterize drug-likeness with respect to chemical structures.[39,40] Both groups used chemical descriptors, training sets from drug collections and from the ACD, and two different versions of supervised neural nets. To some surprise, the trained nets are able to differentiate between drugs and chemicals with a precision of about 75–80%, even if in different runs complete sets of drugs (*e.g.* CNS drugs, cardiovascular

drugs, hormones) were eliminated from the training sets. Whereas a failure rate of 20–25% is acceptable for the evaluation of libraries, it is not suitable to accept or discard a certain compound. Thus, such tools should be used only to enrich or rank libraries and compound collections, and not for individual compounds.

In addition to drug-like properties and drug-likeness, lead structure properties have also been defined, starting from the observation that in recent years the optimization of leads produced most often (much) larger and (much) more lipophilic analogues. Different recommendations were given, most of which restricted molecular weight and lipophilicity to relatively low values.[41–43] However, an independent investigation of 470 lead–drug pairs showed that molecular weight increased, on the average, from the lead to the final drug only by 38 mass units.[44] It was early combinatorial chemistry that misled chemists simply to decorate lead structures with additional rings and other large substituents. Medicinal chemists of the past demonstrated how to modify lead structures in a more intelligent manner, often producing smaller analogues with higher activity or selectivity (*e.g.* several major analgesics, derived from morphine).

How to apply combinatorial chemistry or, better, automated parallel synthesis in medicinal chemistry? Its contribution to lead finding is relatively poor, because of the unfavorable ratio of chemical *vs.* biological activity space, as discussed above. If lead discovery libraries are to be designed:

(i) they should create real diversity by producing many small libraries with different, nonplanar scaffolds (*e.g.* natural products), instead of just one huge library with diverse decoration, and
(ii) the libraries should be checked for their drug-like or lead-like properties and their drug-like character.

The biggest potential of parallel automated synthesis is in chemogenomics and in the early steps of lead optimization. Chemogenomics aims to discover selective ligands of a certain target within a family of proteins or to shift biological activity and/or selectivity from one target to a related one. This is achieved by testing chemically related compounds in classes of evolutionary related targets (GPCRs, integrins, nuclear hormone receptors, aspartyl, metallo-, serine and cysteine proteases, kinases, phosphatases, ion channels, *etc.*).[45] Following this strategy, it is mandatory to synthesize and test a large number of analogues around a lead structure to find directions for further optimization, in any direction. In lead optimization, one should cover the chemical space around the current lead as completely as possible, in order not to lose any interesting candidates and to obtain a solid intellectual property position. Whenever improved candidate molecules are observed (*e.g.* with higher affinity, selectivity, bioavailability, and/or therapeutic range), the process can be repeated around the new structure, if chemically feasible. In the very last steps of lead structure optimization, dedicated syntheses will be necessary – then classic medicinal chemistry is back again.

2.5 Docking and Scoring – Solved and Unsolved Problems

Molecular modeling also had a difficult start. Early limitations in calculation power meant molecules were considered *in vacuo*. However, humans are aqueous systems and all drug targets are surrounded, even "filled", with water. Conformations of molecules were not understood as populations of several to many low-energy geometries – only the minimum energy conformation of a molecule was considered. Slowly chemists and modelers realized that ligand conformations *in vacuo*, in aqueous solution, in the crystal, and at the binding site of a protein may be very different. Whereas it is true that a ligand of a protein will not bind in a high-energy conformation of the ligand and/or the protein, the net free energy of binding results from the balance of entropy gain and entropy loss, as well as enthalpy gain and enthalpy loss. This includes also minor distortions of the ligand and/or the protein, which are to be compensated by other, favorable effects.

Why mention entropy first? The role of entropy is less well understood than the influence of enthalpy and it is most often underestimated. A ligand and its binding site at the surface or in a cavity of a protein are completely covered by water molecules. Some of them are relatively happy, despite the fact that they are more-or-less immobilized (unfavorable entropy), because they form hydrogen bonds to polar groups of either the ligand or the protein (favorable enthalpy). Some other molecules do not feel well, because they are loosely ordered at nonpolar surfaces; there is no favorable enthalpic interaction, only an unfavorable entropic contribution. When the ligand and the protein form a complex, (almost) all water molecules at the interacting surfaces have to be stripped. The water molecules formerly ordered at the hydrophobic surfaces are now happy; they can freely move in the aqueous medium surrounding the complex – this is the driving force of hydrophobic interaction. It is important to know that a perfect fit of hydrophobic residues into their hydrophobic cavities contributes most to ligand affinity; partially filled pockets ("*horror vacui*") or trapped water molecules in such nonpolar surroundings are highly unfavorable.

However, there is never a free lunch, and so also not in ligand binding: if too many hydrophobic groups are present, the solubility of the ligand decreases beyond a level that is acceptable for a drug molecule. The enthalpy terms of the stripping of water molecules, being hydrogen-bonded either to the ligand or to the protein surface, are partially or completely compensated by the entropy gain of their release and the interaction enthalpy of the new hydrogen bond between the ligand and the protein. Instead of increasing affinity, this sometimes results in an unfavorable contribution to binding affinity. In general, hydrogen bonds are important for recognition and for the orientation of a ligand to its binding site, but their affinity contributions are hard to predict.[46] The contributions of hydrogen bonds of the ligand to easily accessible polar groups at the surface of a protein are often overestimated in their affinity-enhancing effect.

In addition to all these effects, there is the unfavorable entropy of freezing the translational and rotational degrees of freedom of the ligand, as well as freezing the internal rotational degrees of freedom of the ligand and the binding site, and the unfavorable enthalpy terms of some distortions of the ligand and/or the binding site.

In his early studies on structure-based design, Peter Goodford developed the computer program GRID, which rolls chemical probes around the surface of a protein to discover regions where certain chemical functionalities should provide favorable interactions.[47] Out of the many interesting applications of the program GRID,[48] probably the most exciting one is the computer-aided design of the neuraminidase inhibitor zanamivir.[49] When Mark von Itzstein applied the program to investigate the 3D structure of neuraminidase, it uncovered a pocket for a positively charged group, close to a hydroxyl group of a weakly active lead structure. With only a few chemical modifications, *e.g.* by exchanging this hydroxyl group with a guanidinium group, the *in vitro* activity of the ligand could be increased by four orders of magnitude – a world record in computer-aided design.[49]

Docking programs go a step further. They use 3D structures of potential ligands to automatically position them into the known 3D structure of the binding region of a target protein. The very first version of the program DOCK[50] considered only geometric complementarity, without searching for potential interactions. LUDI, a hybrid of a docking and *de novo* program,[51] defines interaction sites within the binding site and searches for molecules which have the corresponding functionalities exactly in these positions. In a further step, other small molecules or groups may be attached to such ligands. A simple scoring function was developed to rank the results according to their quality of geometric fit and interaction energies.[52]

Many different programs for rigid and flexible docking and for *de novo* design have been developed in the meantime (for recent reviews see Schneider and Böhm,[53] Schulz-Gasch and Stahl,[54] and Warren *et al.*[55]), most of which generate reasonable poses for the potential ligands. The problem lies in ranking the results – which pose is ''better'' than the others and how can the ligands be ranked according to their estimated affinities? As different docking programs and scoring functions are reviewed in detail elsewhere,[54–60] only the inherent problems of scoring functions are discussed here.

An obvious problem in the calibration and validation of general scoring functions, *i.e.* scoring functions that will be applicable to any ligand–protein complex, is the quality of the biological data. These stem from different laboratories and correspondingly differ in test conditions, precision, and reliability. If one considers the difficulties to reproduce K_i or IC_{50} values (concentration required for 50% inhibition) from one laboratory to another one, standard deviations of one log unit are a reasonable error estimate.

The next problem comes from the protein 3D structures. Even if they are absolutely correct, with respect to electron density interpretation, they often lack a careful inspection and orientation of the hydrogen-bonding groups.

Thus, it might happen that incorrectly oriented hydroxyl groups and asparagine, glutamine, threonine, or histidine side chains, even at a distant site, are responsible for incorrect orientations of amino acid side chains within the binding site. pH shifts may influence the protonation state of histidine and lysine.

Whereas the Protein Database[61] provides many hundreds to thousands of 3D structures of ligand–protein complexes (depending on the desired resolution), it does not contain too many complexes with low, *i.e.* millimolar, ligand affinities. Although such information would be important to study unfavorable interactions, it is under-represented in the calibration of scoring functions. Entropic effects, which are discussed in detail above, are only roughly considered, *e.g.* by the area of the interacting hydrophobic surfaces. Internal rotational energies of ligands and the entropy loss on freezing the bioactive conformation are only considered by a constant term per rotatable bond. Unfavorable geometries of the ligand or the binding site are not considered at all.

Scoring functions do not consider the molecular electrostatic potential of the protein and the dipole moment of the ligand. Inserted water molecules have to be placed "by hand" – whether their replacement is favorable or not can be estimated by the program GRID.[47] Although some docking programs are able to use various conformations of a binding site, the scoring functions do not consider the residual flexibility of the ligand–protein complex. Large ligands have lower affinities than expected from the sum of favorable interactions,[62] an effect that has so far been considered only in some docking studies (*e.g.* Huang *et al.*[63] and Krämer *et al.*[64]).

The importance of desolvation in ligand binding was discussed about 20 years ago, in much detail. In a series of thermolysin inhibitors, a hydrogen bond of the ligands from an –NH– group to a backbone carbonyl oxygen of the protein.[65–68] Replacement of the –NH– group by –O– reduced affinities by three orders of magnitude, which is to be expected because of the lacking hydrogen bond and an electrostatic repulsion between the two oxygen atoms. Replacement of the –NH– group by a $–CH_2–$ group retained affinity,[68] an effect which had already been predicted from modeling two years earlier.[69] The $–CH_2–$ group cannot form a hydrogen bond in the complex, but there is also no negative effect from desolvation, as in the –NH– and –O– analogues.

This is a long list of problems and it is far from complete. Very recently, the group of Brian Shoichet discussed decoys in docking and scoring,[70] *i.e.* molecules with favorable rankings from several scoring functions but without binding affinity. They realized three problems:

(i) scoring functions may tolerate ligands that are too large;
(ii) scoring functions with the hard 12-6 van der Waals potential may miss potential ligands because of steric conflicts;
(iii) scoring functions do not consider the desolvation of the ligands in an adequate manner and therefore overestimate the affinity of polar compounds.

Scoring functions will have to consider such details, otherwise they will continue to fail. In a most comprehensive comparison, GlaxoSmithKline modeling groups at three different locations cooperated to evaluate 10 docking programs and 37 scoring functions against eight therapeutically interesting proteins of seven protein types. In the publication of their results[55] they arrive at the conclusion " . . . no single program performed well for all of the targets. For prediction of compound affinity, none of the docking programs or scoring functions made a useful prediction of ligand binding affinity". Graves *et al.* proposed the use of typical docking decoys as test cases in to further improve scoring functions.[70]

2.6 Virtual Screening – the Road to Success

Virtual screening covers a series of computer techniques, from simple filtering[71] and pharmacophore searches to docking and scoring.[72–77] The title of this section has a double meaning: all virtual screening techniques have to be applied in a proper manner to be successful – if these recommendations are followed, they will be successful in the search for new leads.

Virtual screening starts from a database of real compounds or from a virtual database, in which the chemical structures exist only in the computer. Even at this stage a careful preparation of the database is necessary. Besides the elimination of duplicates and counterions, compounds with undesired functionalities (reactive compounds, organometallics, *etc.*) should be eliminated. If not assigned in a unique manner, all configurations, as well as all enantiomers and diastereomers of chiral compounds, have to be generated. Carboxylic acids should be deprotonated, and amidines and guanidines should be protonated. All other acids (activated sulfonamides, phenols, *etc.*) and bases (amines, nitrogen-containing heterocycles) should be generated, in parallel, in the neutral and ionized forms. Compounds existing as tautomers should be generated in the correct form or as several different tautomers, *e.g.* by the program Agent.[78] 3D structures must be generated if 3D searches or docking are to be performed. If different ligand conformations are not considered "on the fly", multiple low-energy conformations have to be generated. Pharmacophoric features have to be defined in a correct manner, avoiding the attribution of acceptor properties to oxygen atoms with low electron density (*e.g.* the oxygen atom that is attached to the carbonyl group of esters, oxygen atoms in five-membered aromatic heterocycles, *etc.*).[24] All these processes are an absolute must, otherwise pharmacophore searches and docking will fail.

The next steps are options, but they are highly recommended. According to the needs of the user, different filters can be applied to narrow down the originally large size of the database. The filters can set molecular weight ranges, ranges for lipophilicity, and upper numbers for hydrogen bond donors and acceptors (*e.g.* by using the Lipinski rule), but also for polar surface area, number of rotatable bonds, number of rings, maximum number of halogens (more fluorine atoms than chlorine, bromine or iodine atoms may be accepted),

etc. Such filters should be handled with the appropriate flexibility. The next step is the elimination of certain groups that should not be present (*e.g*. multiple amide groups, to eliminate peptides) or the inclusion of groups that should be present (''warhead'' groups, like activated sulfonamides in carbonic anhydrase inhibitors, zinc-complexing groups in metalloprotease inhibitors, *etc*.).

Additional options are filters for lead-likeness, neural nets to prioritize compounds according to their drug-likeness or potential cytotoxicity, pharmacophore models for CYP 450 inhibition, hERG channel inhibition, and other antitarget activities. Too many filters should not be applied at the same time, because they all have a certain error range – thus, using too many might eliminate too many interesting candidate structures.

These preliminary steps are followed by pharmacophore searches. It is appropriate to search first for the presence of the desired pharmacophore groups and only then perform the more time-consuming step of a topological or 3D pharmacophore search. The pharmacophore can be derived from the 3D structures of active and inactive ligands, in a classic manner (*e.g*. by using the ''active analogue approach'') or by appropriate software. If 3D structures of a ligand–protein complex are available, the new program LigandScout is an attractive option to generate a pharmacophore.[79] As an alternative to classic 3D pharmacophore searches, the much faster FTree program may be used,[80,81] which showed the best performance in a recent comparison of different virtual screening protocols.[82] Even higher enrichment factors of active analogues can be achieved by using the newly developed multiple ligand-based MTree approach.[83]

The best candidates from the pharmacophore FTree or MTree searches should be flexibly docked into the experimental 3D structure of the target (beware, 3D structures of unliganded proteins may differ significantly from complex structures; if dimers or oligomers of the protein are the biologically active form, their 3D structures have to be used instead of the monomer 3D structure).

Experience shows that several different scoring functions should be applied to evaluate the docking results – which one will have the better performance for a certain target cannot be predicted *a priori*. Some investigators ''spike'' the database with known actives to find out which scoring function produces the best results. Of utmost importance is a visual inspection of the results for unreasonable ligand geometries, unreasonable binding modes, potential van der Waals clashes, polar interactions at the protein surface, *etc*.

The docking results may be clustered according to their chemical similarity. Only some compounds within a cluster may be picked for biological testing and groups of compounds with already known scaffolds may be eliminated.

A comprehensive review of such virtual screening procedures showed that in almost all cases interesting lead structures were observed, with micromolar to subnanomolar affinities to their target.[77] Even homology models of soluble proteins and GPCRs yielded good docking results, showing the enormous potential of virtual screening in the search for new leads of all potential targets.

2.7 Fragment-based and Combinatorial Design – A New Challenge

In 1975 Green had dissected biotin, the femtomolar ligand of avidin and streptavidin. Elimination of the sulfur atom reduced affinity to some extent. Dissecting this desthiobiotin further, into 4-methylimidazolin-2-one and caproic acid, produced millimolar to submillimolar ligands.[84] A similar effect was obtained by Kati *et al.*, by dissecting a subpicomolar transition state inhibitor of adenosine deaminase.[85] Seemingly, nobody considered to go the opposite way, *i.e.* to design a high-affinity ligand by combining low-affinity fragments.

Only in 1996 did Stephen Fesik develop the SAR-by-NMR (structure–activity relationships by nuclear magnetic resonance) method. This is an experimental approach in which, first, a low-affinity binder for a certain pocket of a binding site is searched for by NMR measurement, then this site is saturated with the ligand and another ligand is sought for an adjacent binding pocket. In the last step, the two ligands have to be linked in a relaxed conformation, to end up with a high-affinity ligand.[86–89] Other experimental techniques followed, based on protein crystallography, NMR, and mass spectrometry (for a comprehensive review, see Erlanson *et al.*[90]). Schuffenhauer *et al.* recently described the design of a dedicated library for fragment-based screening.[91]

The computer program RECAP has been used to dissect drug molecules into pieces that can easily be re-assembled by typical organic reactions.[92] Such fragments can be used for scaffold hopping (*i.e.* to generate a virtual library of new drug-like molecules) by connecting the pieces in a combinatorial manner, and to compare the similarity of the resulting structures with a lead structure.[93] In their search for cyclin-dependent kinase 4 (CDK4) inhibitors, Honma *et al.* first constructed a homology model, starting from the 3D structure of activated CDK2, and then performed a *de novo* design of ligands in the binding site, using the programs LEGEND and SEEDS.[94] Grzybowski *et al.* started from *p*-carboxamidophenylsulfonamide, a submicromolar ligand of carbonic anhydrase.[95,96] Within the binding site of this protein they generated 100 000 different N-substituents of the carboxamido group from a limited number of very small groups, by using the program CombiSMoG (combinatorial small molecular generator). The highest-scoring molecule showed 30 pM affinity. Krier *et al.* propose a scaffold-linker functional (SCF) group approach to convert active ligands into high-affinity analogues. Starting from the phosphodiesterases 4 (PDE4) inhibitor zardaverine, they designed a virtual combinatorial library that combined zardaverine and a few close analogues with different carbon-chain linkers and different functional groups, *e.g.* amines and aromatic rings. The results of this relatively small library were analogues with 40-fold to 900-fold improved inhibitory activity.[97]

These few examples show that the use of computer programs for fragment-based and combinatorial ligand design is just starting (for a recent review see Schneider and Fechner[98]). Programs for incremental flexible docking, like

FlexX,[99,100] could, in principle, be directly used for such a purpose. Instead of the original bits and pieces of the ligand, thousands of alternative structural elements could be used, creating a virtual multitude of potential ligands. These potential ligands need not even be constructed in the computer, and only the best intermediate results of the incremental design would be forwarded to the next step, to assemble the next partial structure (a corresponding tool FlexNovo[101] is in development). However, the problem of ranking the intermediate results is now even much more difficult than that for docking only the original ligand. Further improvements in the scoring functions will be necessary to apply this appealing approach as a routine technique. In addition, the computer programs should include simple rules (*e.g.* the RECAP reactions[92]) for the chemical accessibility of the potential ligands.

2.8 Summary and Conclusions

Within the past few decades the strategies of drug design have changed significantly. Whereas chemistry, biological activity hypotheses, and animal experiments dominated drug research, especially in its "golden age", in the 1960s and 1970s, many new technologies have developed over the past 20 years.[102] A vast number of new drugs was expected to result from combinatorial chemistry and HTS. In the meantime, most groups learned that this is not the case; the yield of new drug candidates was relatively poor and the number of new chemical entities (NCEs) is steadily declining.[103] It is now evident that chemistry-driven syntheses are most often a waste of resources.

Genomics, proteomics, and pharmacogenomics support the discovery of new targets for human therapy. Target validation is performed with genetically modified animals or with the new small interfering RNA (siRNA) technology. System biology and orthogonal ligand–receptor pairs help us to understand the effect of a modulator of a certain protein, long before such a compound is discovered. However, two problems remain: first, will the target be "druggable" (*i.e.* can it be modulated) and will such a modulator be discovered with reasonable effort and within reasonable time? Several protein–protein interactions seem to be not druggable, at least so far. Second, will the modulator of the new target at the very end, after years and years of research, preclinical profiling, and clinical testing, be suited as an efficient and safe drug in human therapy?

Once a target is identified, its 3D structure can be elucidated by structural biology or, at least in many cases, be modeled from the 3D structures of related proteins. With the ongoing progress in protein crystallography and multidimensional NMR studies, the 3D structures of many important proteins, especially enzymes, have been elucidated at atomic resolution. This information enables the structure-based design of therapeutically useful enzyme inhibitors, many of them still in preclinical or clinical development. Whereas structure-based design can be regarded as the predominant strategy of the past decade, several computer-assisted methods were developed more recently. If thousands

of candidates and even larger structural databases are to be tested as to whether there are suitable ligands of a certain binding site, this can no longer be performed by hand. The design process has to be automated, *i.e.* investigated with the help of the computer. Virtual screening selects compounds or libraries that are either lead-like, drug-like, have a good potential of oral bioavailability, or are similar to a lead, by sets of rules, neural nets, similarity analyses, pharmacophore analyses, or docking and scoring.

Correspondingly, the identification of lead structures of a druggable target, by HTS, by structure-based design, or by computer-aided approaches, is now more-or-less a routine approach, as well as their optimization with respect to target affinity. Successful applications of virtual screening, 3D structure-based design, and docking demonstrate the value of these techniques in the selection and rational design of high-affinity protein ligands. Whereas the application of all modern technologies in this step is highly desirable, the risk increases that the accumulated experience in medicinal chemistry[104] becomes increasingly forgotten. Chemists of our time, especially if they lack medicinal chemistry know-how, tend to "decorate" their lead structures, instead of taking the more difficult route of systematic chemical variation, including the formation of new rings (rigidization of a bioactive conformation), replacing ring or chain atoms, *etc.*

The time from selecting a new target to discovering a series of promising leads and optimizing them to nanomolar ligands is now much shorter than in the past. However, this is often accompanied by a neglect of favorable ADME properties. High affinity to a disease-relevant target is only a necessary property of a drug candidate, not a sufficient one. In addition, a drug must have the right selectivity, it must be orally bioavailable, should have favorable pharmacokinetics and metabolism, and should lack serious side effects. The desired degree of selectivity cannot be defined in an absolute manner. In some cases high selectivity is mandatory, in other cases (*e.g.* for kinase inhibitors) a certain lack of selectivity might be tolerable (*e.g.* imatinib, Gleevec™), whereas in the case of CNS-active drugs a high degree of promiscuity might be better than a one-target selectivity.

What are the reasons for the so-called "productivity gap" in pharmaceutical industry, *i.e.* the situation that research costs steadily increase, but output is declining[103]? There is no unique answer and there are no simple reasons. One possible explanation is the already relatively high standard in the symptomatic treatment of "simple", acute diseases. Poor ADME properties are often cited as the most common reason for failure in clinical development, creating a demand for ADME prediction tools; however, this conclusion is based on old data[105] and is not even generally supported by these data.[24,106] ADME became an issue in the attempt to optimize large, lipophilic hits from early combinatorial chemistry and HTS; it was never a major reason for the failure in clinical development, neither in the early period of 1964–1985 (7% attrition rate due to ADME, excluding anti-infectives),[24,106] nor in the years 1992–2002 (11% attrition rate due to ADME).[107] Bioavailability problems can now be minimized at a very early stage, *e.g.* by applying the Lipinski rule.[38]

In our time, the two most common reasons for clinical failure are lack of efficacy and/or toxic side effects; they account for about 75% of all terminated clinical studies[106,107]; both effects cannot be completely separated, because often just the therapeutic window is small – low doses are without sufficient efficacy, but high doses cause toxic side effects. In human therapy, chronic diseases, especially cell-degenerative diseases, are much harder to prevent or treat than acute disorders: often the disease is already too far advanced before it can be diagnosed, *e.g.* in cancer, Alzheimer's disease, or Parkinson's disease. For some progressive diseases it would be desirable to treat healthy people to prevent the development of certain pathological conditions. For this purpose, the drug must be totally free of any side effects, even after treatment over years, which seems to be an impossible task.

Our current expectations on the efficacy and safety of a drug are much, much higher than they were decades ago. Correspondingly, restrictions by the health agencies have increased from decade to decade. It is questionable whether, *e.g.* acetylsalicylic acid (aspirin™) or corticosteroids, would nowadays be approved as "safe" drugs, despite the fact that long-term application shows that they are well tolerated and effective, if applied in the right manner. Rare side effects, which cannot be uncovered in some thousands of animals or by treating a few thousand patients under controlled conditions, end the use of otherwise successful drugs, *e.g.* cerivastatin (Lipobay™) or rofecoxib (Vioxx™).[107]

So, the question is not, "Why aren't we more successful with all these modern technologies?" The question must be, "Where would we stand without genomics, molecular biology, combinatorial chemistry, HTS, structure-based and computer-aided approaches, and virtual screening?" The answer is that the situation would be much worse. Indeed, in the long process of drug discovery and development, from target discovery to launching the new drug, the phase of lead discovery and optimization is nowadays a very fast and most effective one.

How to increase success in drug research? We should merge the know-how of classic medicinal chemistry with the new technologies and follow the recommendations of George de Stevens, who formulated the following as long as 20 years ago. "The (drug) discovery process is at times slow, somewhat tedious, always exciting and requiring patience, tenacity, objectivity and above all intellectual integrity. Therefore, scientists, to be innovative, must work in a corporate environment in which the management not only recognizes these factors but makes every effort to let their importance be known to the scientists. The people in research don't have a need to be loved but they do need to feel that they are understood and supported and not to be manipulated according to short-term business cycles. . . . Drug discoveries are made by scientists practicing good science. By and large these discoveries are usually made in a company with an enlightened management which encourages its scientists with freedom of action, freedom to think widely and to challenge dogma, and freedom in risk-taking. Moreover, important drug discoveries are not made by committees but by individual scientists working closely together, sharing ideas, testing hypotheses, looking for new solutions to difficult problems, accepting

negative results and learning from these results so that the next group of compounds synthesized and tested will open the door to new and improved therapy".[108] This is more true than ever!

[December 2005]

References

1. F. H. Clarke, *How Modern Medicines are Discovered*, Futura Publishing, Mount Kisko, NY, 1973.
2. A. Burger, *A Guide to the Chemical Basis of Drug Design*, John Wiley & Sons, New York, 1983.
3. W. Sneader, *Drug Discovery: The Evolution of Modern Medicines*, John Wiley & Sons, New York, 1985.
4. R. Hirschmann, *Angew. Chem., Int. Ed.*, 1991, **30**, 1278–1301; *Angew. Chem.* 1991, **103**, 1305–1330.
5. T. A. Galemmo, Jr., F. E. Janssens, P. J. Lewi and B. E. Maryanoff, *J. Med. Chem.*, 2005, **48**, 1686.
6. P. J. Lewi, *Arzneim.-Forsch. (Drug Res.)*, 1976, **26**, 1295–1300.
7. C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, 1964, **86**, 1616–1626.
8. H. Kubinyi, *QSAR: Hansch Analysis and Related Approaches*, Volume 1 of *Methods and Principles in Medicinal Chemistry*, R. Mannhold, P. Krogsgaard-Larsen and H. Timmerman, Eds, VCH, Weinheim, 1993.
9. R. D. Cramer III, D. E. Patterson and J. D. Bunce, *J. Am. Chem. Soc.*, 1988, **110**, 5959–5967.
10. H. Kubinyi, *3D QSAR in Drug Design. Theory, Methods and Applications*,ESCOM Science Publishers BV, Leiden, 1993.
11. H. Kubinyi, G. Folkers and Y. C. Martin, *3D QSAR in Drug Design, Volume 2, Ligand–Protein Complexes and Molecular Similarity*, Kluwer/ESCOM, Dordrecht, 1998; also published as in *Persp. Drug Disc. Des.*, 1998, **9–11**, 1–416.
12. H. Kubinyi, G. Folkers and Y. C. Martin, *3D QSAR in Drug Design, Volume 3, Recent Advances*, Kluwer/ESCOM, Dordrecht, 1998; also published as *Persp. Drug Disc. Des*, 1998, **12–14**, 1–352.
13. G. Klebe, U. Abraham and T. Mietzner, *J. Med. Chem.*, 1994, **37**, 4130–4146.
14. H. Kubinyi, F. A. Hamprecht and T. Mietzner, *J. Med. Chem.*, 1998, **41**, 2553–2564.
15. A. Golbraikh and A. Tropsha, *J. Mol. Graphics Model.*, 2002, **20**, 269–276.
16. A. Doweyko, *J. Comput. Aided Mol. Des.*, 2004, **18**, 587–596.
17. H. Kubinyi, in *QSAR & Molecular Modelling in Rational Design of Bioactive Molecules (Proceedings of the 15th European Symposium on QSAR & Molecular Modelling, Istanbul, Turkey, 2004)*, E. Aki Sener and I. Yalcin, Eds., CADDD Society, Ankara, Turkey, 2006, pp. 30–33.
18. J. H. van Drie, *Curr. Pharm. Des.*, 2003, **9**, 1649–1664.

19. J. H. van Drie, in *Computational Medicinal Chemistry for Drug Discovery*, ed. P. Bultinck, H. de Winter, W. Langenaeker and J. P. Tollenaere, Marcel Dekker, New York, 2004, pp. 437–460.
20. S. L. Carney, *Drug Discovery Today*, 2004, **9**, 158–160.
21. R. H. Glassman and A. Y. Sun, *Nat. Rev. Drug Discovery*, 2004, **3**, 177–183.
22. J. Drews, *Science*, 2000, **287**, 1960–1964.
23. A. L. Hopkins and C. R. Groom, *Nature Rev. Drug Discovery*, 2002, **1**, 727–730.
24. H. Kubinyi, *Nat. Rev. Drug Discovery*, 2003, **2**, 665–668.
25. M. A. Fabian, W. H. Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lelias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar and D. J. Lockhart, *Nat. Biotechnol.*, 2005, **23**, 329–336.
26. H. Kubinyi, in *3D QSAR in Drug Design*, Volume 2, *Ligand–Protein Complexes and Molecular Similarity*, ed. H. Kubinyi, G. Folkers, and Y. C. Martin, Kluwer/ESCOM, Dordrecht, 1998, pp. 225–252; also published as *Persp. Drug Disc. Des.*, 1998, **9–11**, 225–252.
27. D. S. Tan, M. A. Foley, M. D. Shair and S. L. Schreiber, *J. Am. Chem. Soc.*, 1998, **120**, 8565–8566.
28. S. L. Schreiber, *Science*, 2000, **287**, 1964–1969.
29. S. L. Schreiber, *Chem. Eng. News*, March 3rd, 2003, 51–61.
30. C. Lipinski and A. Hopkins, *Nature*, 2004, **432**, 855–861.
31. B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, G. F. Lundell, D. F. Veber, P. S. Anderson, R. S. L. Chang, V. J. Lotti, D. J. Cerino, T. B. Chen, P. J. Kling, K. A. Kunkel, J. P. Springer and J. Hirshfield, *J. Med. Chem.*, 1988, **31**, 2235–2246.
32. G. Müller, *Drug Discovery Today*, 2003, **8**, 681–691.
33. C. G. Wermuth, *J. Med. Chem.*, 2004, **47**, 1303–1314.
34. C. G. Wermuth, *Drug Discovery. Today*, 2006, **11**, 160–164.
35. H. Kubinyi, in *Chemogenomics in Drug Discovery. A Medicinal Chemistry Perspective*, ed. H. Kubinyi and G. Müller. Volume 22 of *Methods and Principles in Medicinal Chemistry*, ed. R. Mannhold, H. Kubinyi, and G. Folkers, Wiley-VCH, Weinheim, 2004, pp. 43–67.
36. R. Lahana, *Drug Discovery Today*, 1999, **4**, 447–448.
37. M. Ashton and B. Moloney, *Curr. Drug Discovery*, 2003(8), 9–11.
38. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Deliv. Rev.*, 1997, **23**, 3–25.
39. Ajay, W. P. Walters and M. A. Murcko, *J. Med. Chem.*, 1998, **41**, 3314–3324.
40. J. Sadowski and H. Kubinyi, *J. Med. Chem.*, 1998, **41**, 3325–3329.
41. S. J. Teague, A. M. Davis, P. D. Leeson, and T. Oprea, *Angew. Chem. Int. Ed.*, 1999, 38, 3743–3748 *Angew. Chem.*, 1999, 111, 3962–3967.

42. T. I. Oprea, A. M. Davis, S. J. Teague and P. D. Leeson, *J. Chem. Inf. Comput. Sci.*, 2001, **41**, 1308–1316.
43. J. R. Proudfoot, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 1647–1650.
44. M. M. Hann, A. R. Leach and G. Harper, *J. Chem. Inf. Comput. Sci.*, 2001, **41**, 856–864.
45. H. Kubinyi and G. Müller, *Chemogenomics in Drug Discovery. A Medicinal Chemistry Perspective*, Volume 22 of *Methods and Principles in Medicinal Chemistry*, ed. R. Mannhold, H. Kubinyi, and G. Folkers, Wiley-VCH, Weinheim, 2004.
46. H. Kubinyi, in *Pharmacokinetic Optimization in Drug Research. Biological Physicochemical and Computational Strategies*, ed. B. Testa, H. van de Waterbeemd, G. Folkers and R. Guy, Helvetica Chimica Acta and Wiley-VCH, Zurich, 2001, pp. 513–524.
47. P. J. Goodford, *J. Med. Chem.*, 1985, **28**, 849–857.
48. G. Cruciani, *Molecular Interaction Fields. Applications in Drug Discovery and ADME Prediction*, Volume 27 of *Methods and Principles in Medicinal Chemistry*, ed. R. Mannhold, H. Kubinyi and G. Folkers, Wiley-VCH, Weinheim, 2005.
49. M. von Itzstein, W.-Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, T. V. Phan, M. L. Smythe, H. F. White, S. W. Oliver, P. M. Colman, J. N. Varghese, D. M. Ryan, J. M. Woods, R. C. Bethell, V. J. Hotham, J. M. Cameron and C. R. Penn, *Nature*, 1993, **363**, 418–423.
50. E. C. Meng, B. Shoichet and I. D. Kuntz, *J. Comput. Chem.*, 1992, **13**, 505–524.
51. H.-J. Böhm, *J. Comput. Aided Mol. Des.*, 1992, **6**, 61–78.
52. H.-J. Böhm, *J. Comput. Aided Mol. Des.*, 1994, **8**, 243–256.
53. G. Schneider and H.-J. Böhm, *Drug Discovery Today*, 2002, **7**, 64–70.
54. T. Schulz-Gasch and M. Stahl, *J. Mol. Model.*, 2003, **9**, 47–57.
55. G. L. Warren, C. W. Andrews, A.-M. Capelli, B. Clarke, J. LaLonde, M. H. Lambert, M. Lindvall, N. Nevins, S. F. Semus, S. Senger, G. Tedesco, I. D. Wall, J. M. Woolven, C. E. Peishoff and M. S. Head, *J. Med. Chem.*, 2006, **49**, 5912–5931.
56. I. Muegge and I. Enyedy, in *Computational Medicinal Chemistry and Drug Discovery*, ed. P. Bultinck, H. de Winter, W. Langenaeker and J. P. Tollenaere, Marcel Dekker, New York, 2004, pp. 405–436.
57. E. M. Krovat, T. Steindl and T. Langer, *Curr. Comp. Aided Drug Des.*, 2005, **1**, 93–102.
58. R. Wang, Y. Lu and S. Wang, *J. Med. Chem.*, 2003, **46**, 2287–2303.
59. P. S. Charifson, J. J. Corkery, M. A. Murcko and W. P. Walters, *J. Med. Chem.*, 1999, **42**, 5100–5109.
60. M. Kontoyianni, G. S. Sokol and L. M. McClellan, *J. Comput. Chem.*, 2005, **26**, 11–22.
61. H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, *Nucleic Acids Res.*, 2000, **28**, 235–242.

62. I. D. Kuntz, K. Chen, K. A. Sharp and P. A. Kollman, *Proc. Natl. Acad. Sci. U.S.A.*, 1999, **96**, 9997–10002.
63. N. Huang, A. Nagarsekar, G. J. Xia, J. Hayashi and A. D. MacKerell Jr, *J. Med. Chem.*, 2004, **47**, 3502–3511.
64. O. Krämer, I. Hazemann, A. D. Podjarny and G. Klebe, *Proteins: Struct. Funct. Genet.*, 2004, **55**, 814–823.
65. P. A. Bartlett and C. K. Marlowe, *Science*, 1987, **235**, 569–571.
66. D. E. Tronrud, H. M. Holden and B. W. Matthews, *Science*, 1987, **235**, 571–574.
67. P. A. Bash, U. C. Singh, F. K. Brown, R. Langridge and P. A. Kollman, *Science*, 1987, **235**, 574–576.
68. B. P. Morgan, J. M. Scholtz, M. D. Ballinger, I. D. Zipkin and P. A. Bartlett, *J. Am. Chem. Soc.*, 1991, **113**, 297–307.
69. K. M. Mertz and P. K. Kollman, *J. Am. Chem. Soc.*, 1989, **111**, 5649–5658.
70. A. P. Graves, R. Brenk and B. J. Shoichet, *J. Med. Chem.*, 2005, **48**, 3714–3728.
71. J. A. Lumley, *QSAR Comb. Sci.* 24, 1066–1075 (2005).
72. B. K. Shoichet, *Nature*, 2004, **432**, 862–865.
73. T. Langer and G. Wolber, *Pure Appl. Chem.*, 2004, **76**, 991–996.
74. A. C. Anderson and D. L. Wright, *Curr. Comp.-Aided Drug Des.*, 2005, **1**, 103–127.
75. J. Alvarez and B. Shoichet, *Virtual Screening in Drug Discovery*, CRC Press, Taylor & Francis Group, Boca Raton, 2005.
76. G. Klebe, *Drug Discovery Today*, 2006, **11**, 580–594.
77. H. Kubinyi, in *Computer Applications in Pharmaceutical Research and Development*, ed. S. Ekins, John Wiley & sons, Hoboken, 2006, pp. 377–424.
78. P. Pospisil, P. Ballmer, L. Scapozza and G. Folkers, *J. Recept. Signal Transduct. Res.*, 2003, **23**, 361–371.
79. G. Wolber and T. Langer, *J. Chem. Inf. Model.*, 2005, **45**, 160–169.
80. M. Rarey and J. S. Dixon, *J. Comput. Aided Mol. Des.*, 1998, **12**, 471–490.
81. M. Rarey and M. Stahl, *J. Comput. Aided Mol. Des.*, 2001, **15**, 497–520.
82. A. Evers, G. Hessler, H. Matter and T. Klabunde, *J. Med. Chem.*, 2005, **48**, 5448–5465.
83. G. Hessler, M. Zimmermann, H. Matter, A. Evers, T. Naumann, T. Lengauer and M. Rarey, *J. Med. Chem.*, 2005, **48**, 6575–6584.
84. N. M. Green, *Adv. Protein Chem.*, 1975, **29**, 85–133.
85. W. M. Kati, S. A. Acheson and R. Wolfenden, *Biochemistry*, 1992, **31**, 7356–7366.
86. S. B. Shuker, P. J. Hajduk, R. P. Meadows and S. W. Fesik, *Science*, 1996, **274**, 1531–1534.
87. P. J. Hajduk, R. P. Meadows and S. W. Fesik, *Science*, 1997, **278**, 497–499.
88. P. J. Hajduk, G. Sheppard, D. G. Nettesheim, E. T. Olejniczak, S. B. Shuker, R. P. Meadows, D. H. Steinman, G. M. Carrera Jr, P. A.

Marcotte, J. Severin, K. Walter, H. Smith, E. Gubbins, R. Simmer, T. F. Holzman, D. W. Morgan, S. K. Davidsen, J. B. Summers and S. W. Fesik, *J. Am. Chem. Soc.*, 1997, **119**, 5818–5827.
89. E. T. Olejniczak, P. J. Hajduk, P. A. Marcotte, D. G. Nettesheim, R. P. Meadows, R. Edalji, T. F. Holzman and S. W. Fesik, *J. Am. Chem. Soc.*, 1997, **119**, 5828–5832.
90. D. A. Erlanson, R. S. McDowell and T. O'Brien, *J. Med. Chem.*, 2004, **47**, 3463–3482.
91. A. Schuffenhauer, S. Ruedisser, A. L. Marzinzik, W. Jahnke, M. Blommers, P. Selzer and E. Jacoby, *Curr. Top. Med. Chem.*, 2005, **5**, 751–762.
92. X. Q. Lewell, D. B. Judd, S. P. Watson and M. M. Hann, *J. Chem. Inf. Comput. Sci.*, 1998, **38**, 511–522.
93. G. Schneider, O. Clément-Chomienne, L. Hilfiger, P. Schneider, S. Kirsch, H.-J. Boehm and W. Neidhart, *Angew. Chem. Int. Ed.*, 2000, 39, 4130–4133; *Angew. Chem.*, 2000, **112**, 4305–4309.
94. T. Honma, K. Hayashi, T. Aoyama, N. Hashimoto, T. Machida, K. Fukasawa, T. Iwama, C. Ikeura, M. Ikuta, I. Suzuki-Takahashi, Y. Iwasawa, T. Hayama, S. Nishimura and H. Morishima, *J. Med. Chem.*, 2001, **44**, 4615–4627.
95. B. A. Grzybowski, A. V. Ishchenko, C. Y. Kim, G. Topalov, R. Chapman, D. W. Christianson, G. M. Whitesides and E. I. Shakhnovich, *Proc. Natl. Acad. Sci. U.S.A.*, 2002, **99**, 1270–1273.
96. B. A. Grzybowski, A. V. Ishchenko, J. Shimada and E. I. Shakhnovich, *Acc. Chem. Res.*, 2002, **35**, 261–269.
97. M. Krier, J. X. de Araújo-Júnior, M. Schmitt, J. Duranton, H. Justiano-Basaran, C. Lugnier, J. J. Bourguignon and D. Rognan, *J. Med. Chem.*, 2005, **48**, 3816–3822.
98. G. Schneider and U. Fechner, *Nature Rev. Drug Discovery*, 2005, **4**, 649–663.
99. M. Rarey, B. Kramer, T. Lengauer and G. Klebe, *J. Mol. Biol.*, 1996, **261**, 470–489.
100. B. Kramer, M. Rarey and T. Lengauer, *Proteins Struct. Funct. Genet.*, 1999, **37**, 228–241.
101. M. Rarey, *BIOforum*, 2005, **10**, 56–59.
102. H. Kubinyi, *Technologies*, Vol. 3 of *Comprehensive Medicinal Chemistry II*, ed. D. Triggle and J. Taylor, Elsevier Science, Oxford, 2007.
103. J. A. DiMasi, R. W. Hansen and H. G. Grabowski, *J. Health Econ.*, 2003, **22**, 151–185.
104. C. G. Wermuth, *The Practice of Medicinal Chemistry*, Elsevier–Academic Press, New York, 2003.
105. R. A. Prentis, Y. Lis and S. R. Walker, *Br. J. Clin. Pharmacol.*, 1988, **25**, 387–396.
106. T. Kennedy, *Drug Discovery Today*, 1997, **2**, 436–444.
107. D. Schuster, C. Laggner and T. Langer, *Curr. Pharm. Des.*, 2005, **11**, 3545–3559.
108. G. de Stevens, *Prog. Drug Res.*, 1986, **30**, 189–203.