From Virtual to Real Screening

Achievements and Challenges for Similarity Searching

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The term 'virtual screening' has emerged in the 1990s describing a rather loosely defined set of computational approaches to predicting novel bioactive compounds. Ever since, methods for automated ligand-receptor docking and techniques for molecular similarity searching have emerged as workhorses for computational and medicinal chemists alike to assist in the task of compound selection for activity screening and hit-to-lead optimization (1-6). There is no longer doubt that a smart application and combination of virtual screening tools can actually help find novel chemotypes with a desired pharmacological activity profile (7-9).

While in the early years mere hit identification, library shaping, and rapid database searching was pursued, more advanced tasks are being aimed at today, such as scaffold– or lead-hopping (10, 11), multi-dimensional (multi-objective) optimization of lead structure properties (12), repurposing of known drugs (13, 14), prediction of activity profiles rather than individual targets (15-17), just to name a few. Most importantly, as a main lesson from many virtual screening studies, it has been realized that 'similarity' between two molecules is a context- and target-dependent property, and cannot be expressed by a single universally applicable compound representation or description.

As a general guideline for rapid first-pass similarity searching, it is advisable to represent molecules by shape and pharmacophoric features (Fig. 1). Although this concept has been known for many years, it has been re-discovered for practical virtual screening that these two features capture essential aspects of receptor-drug interactions (18, 19). Ideally, such models implicitly code pharmacologically important molecular properties like lipophilicity. While atomistic and substructure-based molecular descriptors

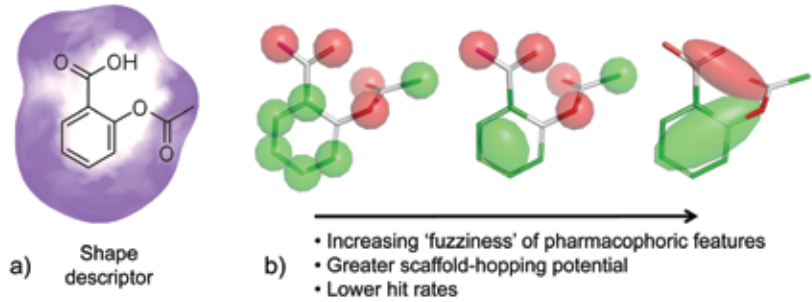

Figure 1. Representation of acetyl salicylic acid by shape (a) and various degrees of 'fuzzy' pharmacophoric points (b). Here, green ellipsoids represent lipophilic centers and red ellipsoids indicate polar, potentially hydrogen-bonding sites. Depending on the degree of abstraction, atomistic models and coarse-grained pharmacophoric models can be obtained. It is important to realize that hit rates in real screening usually decrease with increasing 'fuzziness' of the molecular representation that was used for screening compound selection by similarity searching.

(*e.g.,* 'fingerpints' like MACCS keys (20), Ghose-Crippen fragment keys (21)) are preferred for compound database searching aiming at the rapid retrieval of derivatives of a particular underlying chemotype, shape and pharmacophore descriptors facilitate the identification of bioisosters and scaffold-hops (22-24). It is safe to say that with an increasing abstraction from atom-and-bond (molecular graph-based) models fewer similar but more diverse chemotypes are retrieved from a compound database, which immediately mirrors in reduced hit rates. It is mandatory to realize and accept this dependency, as there are sometimes unrealistically high expectations of hit rates and abundant novel scaffolds among the hits. A weak hit compound featuring a novel scaffold can be a valuable starting point for additional rounds of virtual screening that eventually lead to potent lead structures.

To illustrate the potential of concerted virtual screening, modeling and optimization, an example is presented in Fig. 2. Starting from a known cannabinoid-1 receptor (CB1R) ligand, researchers at Roche employed pharmacophore-based de novo design for scaffold-hopping and first hit identification (25), which was followed by further refinement by pharmacophore compliance, chemical tractability, and drug-likeness considerations, and eventually resulted in a series of benzodioxoles as potent novel CB1R inverse agonists. This strategy of extensive multidimensional optimization involved tight interaction between virtual and real screening and was rewarded by the identification of promising lead compounds with significant in vivo activity (26).

Another hallmark example of broad virtual screening and similarity searching in tight combination with real screening has recently been given by Roth and coworkers who found several new targets for marketed drugs and conclude that *"The chemical similarity approach is systematic and comprehensive, and may suggest side-effects and new indications for many drugs."* (14)

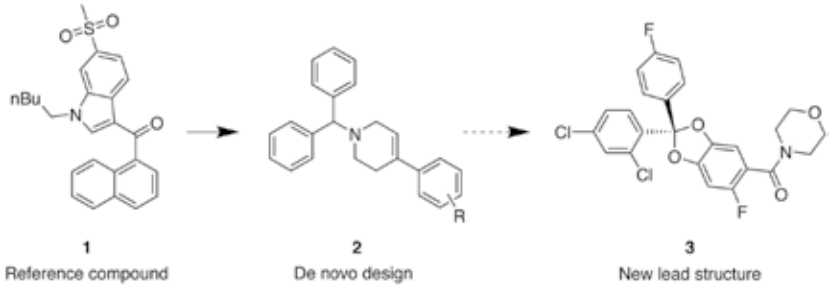

Figure 2. Example of a strategy for multidimensional optimization by iteratively applied virtual screening methods. Starting from the known cannabinoid-1 receptor (CB1R) effector **1** (K_i = 0.11 µM) (51) as a reference compound, fragment-based *de novo* design with the software TOPAS (52) led to weakly active hits **2** in small combinatorial libraries that were derived from design. Using these hit compounds as queries for pharmacophore-based similarity assessment and subsequent medicinal chemical optimization finally resulted in lead compound **3** (K_i = 0.03 µM), a potent CB1R agonist featuring a novel scaffold compared to the original reference **1**.

Guidelines for fast and successful similarity searching

There are some basic guidelines for first-pass virtual screening by *similarity searching* that may be deduced from various recent research reports. These tips should be understood as empirical findings (supported by personal experience and preferences) rather than analytically obtained rules:

- Use all potent reference compounds as queries. This will increase the chance of finding potent hits (27).
- Describe molecules by their topology or shape plus pharmacophoric features to capture two complementary molecular features that are generally relevant for ligand-receptor interaction (23, 24).
- Whenever possible, describe molecules by few features (28). This will help avoid artifacts resulting from high-dimensional compound descriptions (the "curse of dimensionality") (29).
- For each of the molecular representations perform individual similarity searches in large compound collections (30). This will help increase the number of different chemotypes among the top-scoring compounds.
- Compute pair-wise molecular similarity by two complementary techniques: a *metric* (*e.g.,* Manhattan distance, Euclidian distance) and a *similarity index* (*e.g.,* Tanimoto-index) (29). This can help avoid artificial bias towards certain chemotypes.
- Combine individual ranked compound lists, *e.g.* by 'group fusion' (31, 32).
- Trust the results and consider the top-scoring compounds for biochemical testing, irrespective of your own or your colleagues' opinion.

- Look at the top-scoring compounds and select additional screening candidates manually.

In any case, at least two subsequent rounds of virtual and real screening should be performed before an assessment of the overall outcome of the screening campaign should be made. Numerous studies have shown that initial weak hits can be optimized to potent ligands in subsequent select-and test cycles. It is always a good idea not to blow all screening capacity in one shot but to learn in iterative steps. This can be done, for example, by using initially poor hits as queries for a second or third virtual screening triage (33).

Notably, a description of 'two-dimensional' molecular structure (in particular descriptors of molecular topology) has repeatedly led to surprisingly good results. One reason might be that three-dimensional representations by shape and pharmacophores as well as molecular docking methods have to explicitly cope with conformer generation and flexibility, while some of the available topological descriptors have the ability to implicitly capture relevant aspects of conformational preferences and restrictions (34, 35). This does by no means render three-dimensional approaches like docking useless but pinpoints to a general issue of virtual screening protocols. Recently, Bajorath and coworkers successfully transferred information about relevant receptor-ligand interaction sites from three-dimensional binding complexes to two-dimensional fingerprints, which resulted in improved hit rates (36).

What's next?

Ignoring various technological aspects, publication policies, the 'human factor' and obvious project-specific considerations, it is fair to say that a main achievement of virtual screening as a whole has been to provide methods that help eliminate the bulk of inactive compounds, rather than actually predict actives (37). While this statement is certainly simplifying (and grossly inadequate for individual studies and techniques) it pinpoints to one of the major limitations we are currently facing, namely the *accurate and rapid* quantitative computation of binding free energies (respectively pK_d, pIC_{50}) for arbitrary ligand-receptor complexes (38). This poses a problem not only for three-dimensional receptor-ligand and protein-protein docking (scoring problem (39, 40)) but also for advanced QSAR techniques and machine learning approaches. While there are elegant and analytically exact algorithmic solutions for compound placing in a binding site and mathematical model finding and optimization, we still lack sufficiently accurate approximations of basic physical and chemical phenomena of receptor-ligand binding or fail to model them with sufficient accuracy within a reasonable time. These include in particular

- entropic contributions to ligand binding
- dynamic behavior of both ligands and macromolecular targets
- role of solvent (water, ions) in complex formation and stability

- dynamic conformational ensembles of ligands and ligand-receptor complexes.

Explicit consideration of the flexibility of ligands and receptors in docking and pharmacophore modeling has already led to some progress. For example, ligand-based virtual screening by *pseudo*-receptor models (41) was combined with molecular dynamics simulations to obtain ligand binding sites in comparative protein models ('homology' models) of the histamine H4 receptor, a G-protein coupled receptor (42). Comparative protein models have also gained attractiveness for ligand docking and scoring during the last year due to developments in consensus scoring and flexibility considerations (43). DOCK Blaster is an expert system for unsupervised high-throughput ligand docking with multiple targets that re-assesses the correctness of ligand binding poses in three-dimensional protein models (44). Another innovative concept was realized in the HYDE scoring function, namely the use of log*P*-derived atomic increments for the computation of free dehydration and hydrogen bonding energies, which resulted in improved accuracy and in particular fewer false-positives (45).

While these approaches focus on complex scoring and molecular flexibility, other seemingly trivial problems remain largely unsolved until now, for example the accurate prediction of tautomerization equilibria (46) or computation of pK_a values (47, 48).

Developments in computer hardware have always led to an improvement of modeling capabilities. We are currently witnessing an exciting development of specialized graphics processor chips and parallel computing technology as potential workhorses for molecular dynamics and other computationally very demanding calculations (49, 50). No doubt this will enable long time (beyond 100 ns) dynamics simulations of macromolecular targets on a routine basis, which can help understand allosteric effects, flexible fit phenomena of druglike ligands and effector molecules (51). As a consequence, dynamic molecular features (in contrast to 'static' properties like molecular mass, log*P*, and other time-invariant molecular properties) that cannot be accurately modeled today, will be within our grasp and improve the accuracy of future predictions of novel bioactive compounds.

Acknowledgements

I am very grateful to Herbert Köppen, Tim Clark, Nikolaus Heinrich, and Karl-Heinz Baringhaus for stimulating discussion of the myths and facts of virtual screening.

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