Synergies of Virtual Screening Approaches

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Abstract: Virtual screening is a knowledge driven approach. Therefore, synergies between different virtual screening methods using information about the drug target as well as about known ligands in combination promise the best results. Finding novel active scaffolds is often a more important success criterion than hit rates of virtual screens. Novelty should also be considered in balance with often weaker activities of virtual screening hits. Virtual screening is most effective if performed in iterations following up on weak primary hits of interest through testing of structural analogs and additional synthesis of compounds.

Key Words: *In silico* screening, docking, scoring, compound similarity, pharmacophore.

INTRODUCTION

Virtual screening (VS) can be viewed as *in silico* equivalent to high throughput screening (HTS) [1]. It is a knowledge driven compound database searching approach that attempts to find novel compounds and chemotypes as alternatives to existing ligands [2] or sometimes to make first inroads into finding ligands for previously unexplored putative drug targets for which crystal structures, solution structures, or high confidence homology models are available [3]. VS is usually described as a cascade of filter approaches to narrow down a set of compounds to be tested for biological activity against the intended drug target [4,5]. Depending on the intended follow-up (testing of available compounds or synthesis of VS hits before testing) databases for VS contain between up to ~10 million available compounds and any number of virtual compounds the VS approach can handle (10^{12} compounds is often the limit). Starting with a fast evaluation of the drug-likeness of compounds [6], VS is often followed by ligand-based approaches including 2D and 3D similarity to known active molecules including 2D and 3D pharmacophore approaches and subsequent structure-based screening using docking and scoring approaches [7] if the target structure is available. The merits of ligand-based VS (LBVS) [8,9] and structure-based VS (SBVS) [10-12] approaches have been discussed in the literature independently for a long time. In the past researchers have sometimes assumed that SBVS as the computationally more expensive technique is also more powerful in finding novel active molecules compared to LBVS approaches. Therefore, LBVS approaches have been attempted less often if SBVS techniques have been available and have looked promising. This perception has been challenged now. Quantitative comparisons have been published in recent years illustrating that in many cases LBVS is as powerful or sometimes even outperforms SBVS approaches across a series of different drug targets [8,13-15] while in other cases SBVS is still superior [16].

The main goal of VS approaches is to find new actives of novel chemical structure. While for LBVS approaches the ability to 'scaffold hop' (finding new hits of novel chemotype) has always been in the center of the studies, finding new chemotypes in SBVS campaigns has often been taken for granted. This may be the reason that only very limited studies have been published on this topic [17]. Reviewing published VS results in the context of comparing LBVS and SBVS methods identifies the need to quantify scaffold hopping capabilities of docking approaches. Overall, recent studies comparing LBVS and SBVS approaches, especially in the context of scaffold hopping, show that the knowledge of active small molecules is as useful as and sometimes even more useful than the knowledge of the structure of the target protein at atomic resolution [8,15]. However, another important observation comparing LBVS and SBVS approaches is that both methods identify different sets of novel actives. This finding suggests that LBVS and SBVS should not be used in cascade but rather in parallel complementing each other in the hunt for new hits.

VIRTUAL SCREENING AS SCAFFOLD HOPPING TOOL

Similarity approaches have been used for a long time to find new biologically active compounds based on their similarity to known ligands [18]. Focusing on compounds with high similarity to known actives it has been found that there is only a 30% chance that compounds with high topological similarity measured by a Tanimoto coefficient of more than 0.85 using Daylight fingerprints as descriptors are active also against the same drug target [19]. These compounds are structurally very similar to active template molecules. On the other hand, there are examples of identifying novel actives based on compounds that are topologically quite dissimilar to the template ligands, a task that is obviously far more challenging. An example is the identification of a 15-LO inhibitor through 3D pharmacophore fingerprint similarity-based virtual screening (Fig. **1**) [20]. Although the Tanimoto similarity between the template molecule **1** and the VS hit **2**

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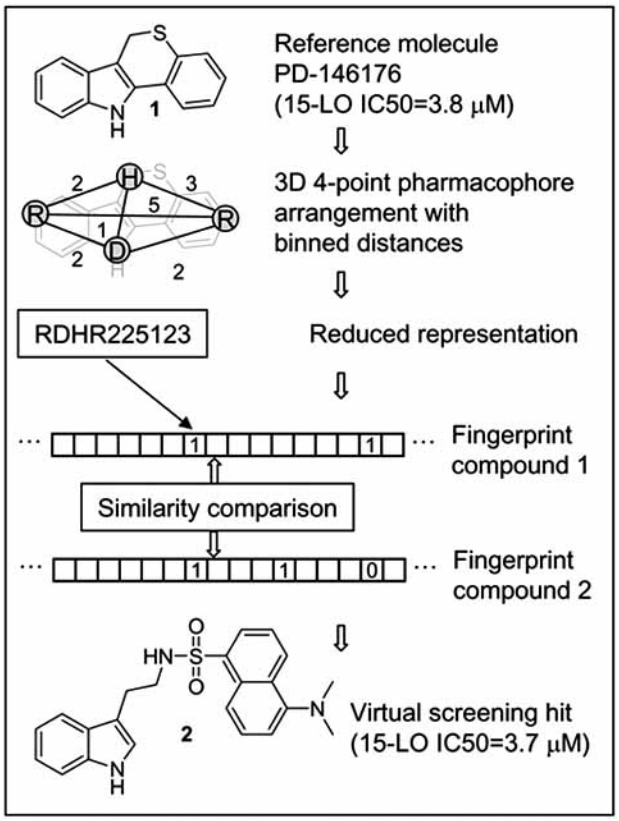

Fig. (1). Discovery of a 15-LO inhibitor through virtual screening using a 3D pharmacophore fingerprint similarity technique [20].

using Daylight fingerprints is only 0.41 (Scitegic fingerprint FCFP4 descriptor similarity is only 0.33) the compounds appear to be equally potent. How can this be? For a virtual screen in which active compounds of any scaffold type exist in the database of molecules subjected to the screen, it is not the question, how closely related these compounds are to the known actives but rather if they are significantly more similar to the actives than to all other inactive compounds in the data set screened. The task at hand is to find a relevant descriptor that associates such higher similarity scores with unknown actives thereby separating them from the majority of inactive compounds subjected to the virtual screen. In other words, the similarity to known actives can be low as long as the similarities of other inactive compounds are even lower. Obviously, there has to be a threshold for minimum similarity below which noise takes over. However, this threshold is far lower than the Tanimoto similarity of 0.85 using Daylight fingerprints cited above. On the other hand, the 15-LO example (Fig. **1**) also illustrates that VS hits based on low similarity are sometimes obtained serendipitously. The similarity between the 2 very different compounds **1** and **2** (template and VS hit) is most likely driven by the common indole substructure. Inspecting probable binding modes of compounds **1** and **2** in a high confidence homology model (data not shown) reveals, however, that it is unlikely that the indole moieties are placed similarly in the 15-LO binding pocket suggesting that the successful activity prediction of **2** based on similarity to **1** may have been all for the wrong reasons. This example illustrates that while VS hits are certainly welcome by a drug discovery team, a careful evaluation of the underlying computational approach is nevertheless warranted to draw more general conclusions about the merit of a particular VS approach.

There are generally some sets of descriptors that tend to be better suited to find actives of novel scaffolds than others. Ultimately, for a given target, however, it is necessary to test several descriptors using sets of known actives as templates to determine the optimal combination of descriptors to be applied. Ideally one can combine ligand-based descriptors with structure-based descriptors as they often tend to be orthogonal to each other. Here are some observations on the ability of different descriptors to scaffold hop. In a study of seven drug targets for which crystal structures are available in the literature as well as a selection of known ligands of different chemical classes is known, the ability of several descriptors to scaffold hop has been analyzed. Fig. **2** shows how atom pair descriptors (AP), 3D pharmacophore fingerprints (P50), and Scitegic fingerprints (ECFP4) perform in retrieving known actives of different chemotypes from databases of decoys. As described in detail elsewhere [15] AP and pharmacophore fingerprint methods are able to facilitate scaffold hopping well. Recently, we have found that Scitegic fingerprints (ECFP and FCFP) are also very strong in finding new chemotypes through virtual screening (data not shown). Especially in case of estrogen receptor and thrombin ligands, Scitegic fingerprints have performed very well in hopping from one ligand chemotype to another.

CONSIDERATIONS OF DATABASES TO BE SCREENED

Depending on whether the database of compounds deployed for VS experiments contains actives that are topologically related to the query compounds - perhaps through similar positioning of pharmacophore-bearing functional groups on side chains that are not part of the scaffold - the choice of optimal descriptors need be different. In cases where similarity to known actives can be established through non-scaffold related topological similarities, topological descriptors such as Daylight fingerprints, AP, or Scitegic fingerprints, and many other topology-dependent approaches perform very well. This is often the case when recall experiments using literature compounds are designed pooling known actives from different scaffold types with a database of inactives. Because there is often correlation among the active molecules through historic dependencies of their discoveries, scaffold hopping seems often easier to be accomplished. Fig. (**2**) shows examples where such bias exists. For the drug targets HIV-1 protease, p38 kinase, thrombin, and estrogen receptor, known actives of different scaffolds have been mixed with perceived inactive drug-like molecules from the MDDR database and then recalled using different descriptors as basis for similarity assessments. As one can see from the figure, topological fingerprints perform well in this exercise of scaffold hopping. Scitegic fingerprints perform particularly well. However, in cases where such topological bias does not exist, such as for the CDK2 case, topological fingerprints have a much harder time to compete with other descriptors such as 3D pharmacophore fingerprints or docking methods that depend much less on topological similarities [15].

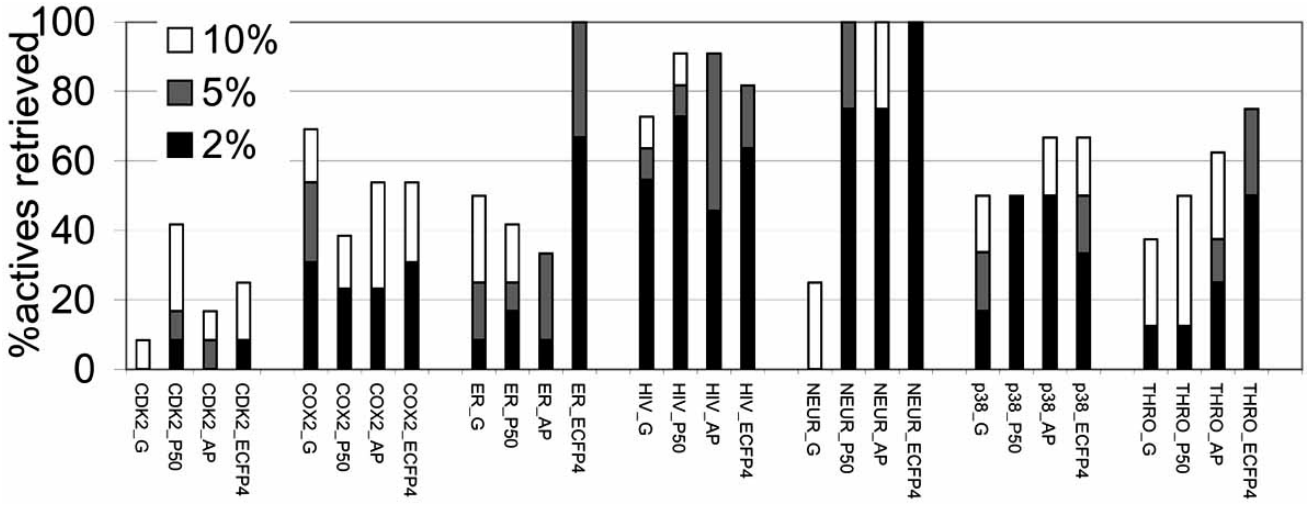

Fig. (2). Comparison of percent actives retrieved in the top ranking 2, 5, and 10% of a database in a recall experiment involving seven protein targets (CDK2, COX2, estrogen receptor, HIV-1 protease, neuraminidase, p38 MAP kinase, and thrombin [15]. The suffix _G refers toGlide2.5 docking experiments. _P50 and _AP refer to ligand-similarity VS methods using 3D pharmacophore fingerprints with 50 conformations per molecule and to atom pair descriptors, respectively. _ECFP4 refers to Scitegic fingerprints. Between 4 and 13 active ligands are involved per target paired with 9969 putative decoys from the MDDR database [39].

It is important for each similarity-based VS to assess whether the chances of finding structurally related compounds (for instance through common pharmacophore-bearing side chain similarity) are high or low. If chances are high, topological descriptors, especially Scitegic fingerprints, will perform well in finding compounds with novel scaffolds. If the chances are low, pharmacophoric descriptors may be an alternative. A more relevant assessment of the power of different descriptors should be obtained from the analysis of HTS data. VS recall assessments are often less relevant because of the built-in correlation within historic data sets. Compounds in the MDDR database, for instance, with activities against the same target, are often the result of literature-to-lead approaches and structurally very similar to each other. Such high compound similarities are often unlikely to be found in unbiased screening collections. It should also be mentioned that the success of virtual screening experiments depends critically on the inactive compounds in the database screened against [21,22]. The occurrence of false positives (inactive compounds predicted as active) is one of the main problems in VS and is obviously very dependent on 'what else' is in the database to be screened.

COMPARING STRUCTURE-BASED AND LIGAND-BASED VIRTUAL SCREENING APPROACHES

Recently, several reports of direct comparisons of structure-based and ligand-based virtual screening approaches have been reported in the literature. Evers *et al*. have compared molecular docking into GPCR homology models with ligand-based pharmacophore models, Feature Tree models [23], statistical methods including partial least square (PLS) and PLS discriminant analysis based on 2D descriptors [24]. Four aminergic GPCRs (Alpha1A, 5HT2a, D2, and M1) with a variety of ligands of different chemotype have been used in a virtual screening recall study. While homology models, especially for GPCRs, generally introduce an additional uncertainty into structure-based virtual screening studies [25,26], the authors have validated at least one of the models (Alpha1A) through experimentally testing structure-based virtual screening hits finding 37 novel antagonists [27]. The authors have carefully selected a set of 42-48 unique chemotypes among 50 active ligands chosen for each receptor combined with 950 inactives from the MDDR that are purged of biogenic amine binding moiety containing compounds. For the described setup, the ligand-based methods have yielded remarkable enrichment rates among the recalled actives in a virtual screen. Particularly the Feature Tree and PLS approaches have performed well. Structure-based approaches (GOLD docking [28] and FlexX docking [29]) have performed less well; however, they still provide satisfying enrichment rates (up to 60% of actives found in the top 1% of the screened database). The authors stress the point that "the chance of being successful in virtual screening increases if different virtual screening approaches are employed in parallel or in combination with each other".

In our own work we have also compared structure-based and ligand-based VS methods [15]. As shown in Fig. (**2**), ligand-based virtual screening performs sometimes better than molecular docking (here using Glide [30]). While this finding may be surprising at first it may again be a reflection of the strong topological bias included in the particular recall data sets used that have resulted in a particularly strong performance of topological descriptors. On the other hand it also points towards opportunities in combining the different approaches to increase the predictive power of VS.

SYNERGIES BETWEEN STRUCTURE-BASED AND LIGAND-BASED METHODS

Similar to the work of Evers and coworkers described above, Bissantz *et al*. [14] have compared virtual screening performances of different ligand-based methods (Feature Trees, Phacir, Daylight) applied to GPCR homology models

with docking and scoring (FRED, FlexX). Four different 5HT2c agonist structures of different chemotype have been used (Fig. **3**) in a recall study involving 207 active molecules (40% functional activity) among a set of 9,955 compounds selected to have a higher chance of being aminergic GPRC ligands. In contrast to the decoy set used by Evers at al. that does not contain any potential biogenic amines, the decoy set Bissantz *et al*. use possesses exactly one basic amine moiety, at least one aromatic ring, no negative charge, and each molecule has a molecular weight of less than 400 Da. Not surprisingly and in stark contrast to the work by Evers at al., the hit rates obtained with all approaches are not particularly high because of the functional similarity of the compounds although diversity criteria have been applied to choose the final selection of compounds to screen against. Despite the differences in the works of Evers and Bissantz the most interesting observation of the Bissantz paper is again the complementarities of the actives found with different approaches. On average the overlap between a particular ligand based approach (using one of the 4 reference ligands as templates) and a structure-based approach (FlexX or FRED docking with different scoring functions) is only about one third while the majority of the hits are either identified by the ligand-based or structure-based approaches. Also noteworthy is the finding that while the hit rate has been often higher for ligand-based approaches, the diversity of hits and the stability of the hit rate independent of the virtual screening protocol has been higher for the structure-based approach.

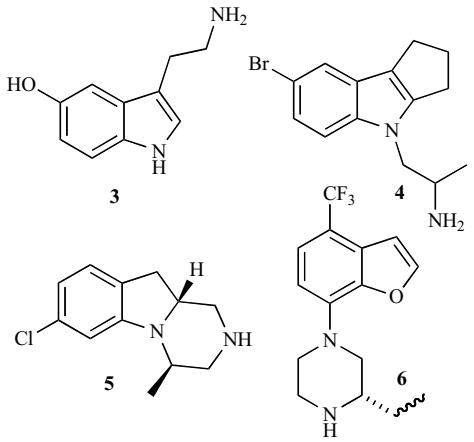

Fig. (3). 5HT2c agonists used in a VS study by Bissantz *et al*. [14].

There are developments to combine SBVS and LBVS approaches in software solutions. While developed for improving docking mode predictions, the SDOCKER concept [31] of combining ligand similarity as additional force in docking experiments can be used for VS applications also. Commercially available docking programs such as Glide also allow for compound similarity to be considered in molecular docking [30].

USING LIGAND-BASED AND STRUCTURE-BASED METHODS IN CONCERT

The concept of using different virtual screening approaches in concert is an obvious choice. More researchers try to incorporate multiple VS screening methods as part of a comprehensive VS strategy. Table **1** provides an overview of some of the work that has been reported in the literature in recent years. The selection shown in the table is not exhaustive, of course. It has been assembled to merely illustrate the variety of approaches and target classes pursued with synergetic VS concepts to date. Combinations of docking approaches, pharmacophore screening, shape, similarity, 2D and 3D similarity, clustering methods, QSAR, and machine learning methods are prevailing in several publications aiming at increasing the effectiveness of VS. A few of these efforts are briefly described below.

A recent example of how VS methods can be combined successfully has been reported by Hu and coworkers [32]. The first Yersinia Protein Kinase A (YpkA) inhibitors with activities in the single digit μM range have been reported through the combination of support vector machines (SVM) and ensemble docking using a multitude of homology models. Fig. (**4**) illustrates the conceptual setup of the VS. First, a 2 million compound database has been filtered down 10-fold using a general kinase inhibitor trained SVM approach. Then the surviving compounds have been docked followed by consensus scoring against multiple YpkA homology models.

A surprising number of recent successes in VS have been reported using homology modeling for GPCRs. While there are examples in which different ligand-based techniques used in concert have allowed for synergies to be gained all three GPCR examples in Table **1** used docking techniques against GPCR homology models as part of a VS strategy in one form or another. An example of finding potent antagonists against melanin-concentrating hormone (MCH) 1R receptor has been described by Clark *et al*. [33]. Substructure searching, 2D and 3D similarity searching have been used in concert and have been followed by manual docking into a bovine rhodopsin-derived homology model. Similarly, successes have been reported combining similarity and homology model docking methods for GPR30 [34] and CCR5 [35].

An interesting example of how different VS methods can yield different complementary hit sets is given by Pirard *et al*. [16]. A protein-based pharmacophore model has been derived based on homology models of the potassium channel Kv1.5. VS using this model has yielded 19 potent inhibitors from five distinct chemical classes. In comparison, independent 2D similarity searches using UNITY fingerprints have yielded two hits from two classes and an unrelated 3D ligand-based pharmacophore searching VS approach has provided one hit. While for the potassium channel virtual screen the protein-derived method has worked best it is particularly noteworthy that there has been no overlap observed between the hits or chemical classes found with the three individual methods mentioned above. This is an excellent example of how different methods - in this case protein and ligand-based 3D pharmacophore methods and a 2D similarity approach) - are able to provide complementing hits that would otherwise have possibly been ignored.

CONSENSUS SCORING IN VIRTUAL SCREENING

Particularly in SBVS the use of unreliable scoring functions has been cited as main reason for less robust VS results. Attempts have been made to overcome this problem

Table 1. Targets Pursued using Multiple Virtual Screening Strategies in Synergy

Target Class	Target	VS Approaches Used	Outcome	Reference
kinases	YpkA	machine learning and multiple conformational virtual screening	first reported single digit μM inhibitors	Hu *et al.*, 2007 [32]
	EphB2	docking and scoring, pharmacophore searching	μM inhibitors	Toledo-Sherman *et al.*, 2005 [40]
G-protein-coupled receptors	MCH-1R	2D,3D similarity, substructure searching, manual docking, clustering	55nM hit with antagonistic properties	Clark *et al.*, 2004 [33]
	GPR30	2D similarity, pharmacophore and shape-based similarity, docking	first GPR30-specific agonist	Bologa *et al.*, 2006 [34]
	CCR5	2D pharmacophore similarity, docking, clustering	agonist found that promotes receptor internalization	Kellenberger *et al.*, 2007 [35]
proteases	SARS protease	eHITS docking, 2D similarity	testing of hits ongoing	Plewczynski *et al.*, 2007 [41]
	SARS protease	docking, 3D-QSAR, pharmacophore	25 inhibitors >3μM	Tsai *et al.*, 2006 [42]
ion channels	Kv1.5	protein-derived pharmacophores, FeatureTrees, 2D-similarity	5 inhibitor classes <10μM	Pirard *et al.*, 2005 [16]
nuclear receptors	PPARg	shape similarity, docking, analog searching	novel PPARγ agonists	Lu *et al.*, 2006 [43]
other enzymes	chorismate mutase	ligand-based pharmacophore models, docking	6μM inhibitors	Agrawal *et al.*, 2007 [44]
	DPPIV	pharmacophore searching, docking	51 inhibitors found	Ward *et al.*, 2005 [45]
	COX-2	machine learning, pharmacophore descriptor	new inhibitors found	Franke *et al.*, 2005 [46]
protein-RNA interactions	HIV-1 reverse transcriptase	3D-QSAR, docking	2 potent inhibitors from SPECS	Zhang *et al.*, 2006 [47]
transporters	SHGB	2D-QSAR, docking	ligands found	Cherkasov *et al.*, 2005 [48]
others	HRV coat protein	structure-based pharmacophore model, docking, PCA	6 structures with antirhinoviral activity	Steindl *et al.*, 2005 [49]

using consensus scoring approaches [36]. Most consensus approaches use an average principle of multiple scoring functions. However, recently, some more sophisticated methods have been used ranging from clustering ideas [15] to supervised learning methods [37]. Learning methods may be a good choice if the performance of multiple individual screening functions is uneven. In other words, simple combinatorial approaches work only satisfactorily if all scoring functions are equally predictive. If a well performing scoring function is combined with underperforming scoring functions consensus scoring without learning will not improve results beyond the predictive power of the best scoring function. We have seen this behavior for instance in case of LBVS combining different similarity descriptors and have found only one case in which clustering-inspired consensus scoring improved results beyond those obtained with the best individual method [15]. However, generally, an averaging effect is observed through the use of consensus scoring and may be preferred even in cases that scoring functions perform quite differently because for a given virtual screen it may be unknown which scoring function performs better than others. This observation holds true for both SBVS and LBVS methods.

WEAK HITS – WHAT TO DO WITH IT?

A common observation in VS experiments is the finding of rather weak hits in the μM range (often double digit) [38]. Especially for advanced projects looking for novel starting

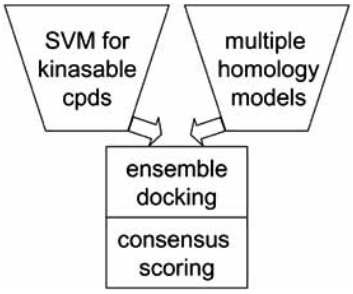

Fig. (4). Successful combined machine learning and structure-based virtual screening strategy to identify first YpkA inhibitors [32].

points for back-up series these hits often appear less attractive. However, even weak VS hits are often complementary to HTS hits and structurally different. Because VS is not an exact science weak hits with interesting structure should be seen as door openers to a potentially new world of chemicals that could be relevant to the target at hand rather than the ultimate set of best compounds available. Both false negatives (active compounds predicted to be inactive) and false positives cloud the picture of VS results. Therefore, VS experiments need to be done in iterations. At the least hits should be followed up with the testing of available analogs. Even better is a synthetic exploration of hits that sometimes can be most effectively achieved using combinatorial chemistry. In many cases analog testing improves potencies significantly.

SUMMARY

Using multiple approaches in concert is now a common strategy in virtual screening. Interestingly, combining LBVS and SBVS is often practiced in cases of low confidence in the target structure, most notably for GPCRs where homology models have been reported to be used in VS combined with different compound similarity approaches. While the desire for GPCR targets to use multiple methods is most likely driven by the need to use all the information one can get in an often information-poor environment, the strategy of combining structure-based and ligand-based approaches should be generally adopted to realize maximum VS potential even if target crystal structures are available.

Pursuing virtual screening results needs a sustained commitment for testing follow-up compounds. Primary results often only open first doors by providing weak hits. VS works best in iterations learning from and following-up on first round results. While this is common practice for VS in an industrial setting, specific reports on such iterative VS setups in the literature are still sparse. While synergies between different virtual screening methods are more often realized there is a need to embed VS better in the drug discovery process and realize synergies with other drug discovery functions, especially with biomolecular screening and medicinal and combinatorial chemistry.

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