

# 3D pharmacophores as tools for activity profiling

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**The parallel use of multiple pharmacophore models representing different pharmacological targets emerges as an *in silico* tool for compound activity profiling. This technology allows for the prediction of desired bioactivities together with potential adverse effects of a drug candidate. In the field of ethnopharmacology, activity profiling can guide the rationalization of traditional drug uses and the discovery of their active principle. This article highlights the concept, recent applications and caveats of pharmacophore-based activity profiling.**

## Introduction

Pharmacophore-based virtual screening is an established *in silico* tool used for filtering large compound libraries in the search for novel lead compounds [1]. A pharmacophore model may represent a target-ligand binding site that triggers a desired pharmacological effect. Alternatively, such a model may also hold information of chemical features that can lead to adverse drug reactions or drug–drug interactions. Thus, when screening a compound against a panel of models representing multiple pharmacological targets, an activity profile including desired and unwanted actions of the compound can be predicted (Fig. 1). Subsequent *in vitro* and *in vivo* experiments verify or falsify the *in silico* prediction. Activity profiling is therefore a means of selecting the most promising compounds from a database for biological testing. Applications range from screening for lead compounds over natural product profiling to environmental chemicals assessment.

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## Set-up of a pharmacophore model collection

The heart of pharmacophore-based activity profiling consists of numerous pharmacophore models representing a set of pharmacologically relevant targets, the so-called pharmacophore model collection (PMC). As the quality of any method is heavily dependent on the quality of its single components, each of these pharmacophore models needs to have good predictive power. True/false positive hit rates, true/false negative hit rates, enrichment factor, goodness of hits, or the receiver operating characteristic curve–area under the curve (ROC–AUC) are among the most common quality parameters used in model evaluation experiments [2]. As another aspect, the purpose of each pharmacophore model has to be defined. A model that is intended for cherry-picking of promising candidates for biological testing may require a higher enrichment of actives in the hitlist than a model for potential adverse drug reactions, which aims to broadly identify suspicious compounds. In the first case, the model is not supposed to cover all active compounds from a database; however, it is required that the model does not return too many false positive hits from a database search. In the second case, models can be more general in order not to miss any compounds that might trigger undesired effects. Thus, false negative hits are to be avoided. As a consequence, when a PMC is aimed at broad and multi-purpose activity profiling, hundreds of models of different qualities are required. One pharmacological target may be represented by several models

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## Glossary

**Enrichment:** enrichment of active compounds in the virtual screening hitlist in comparison to the non-filtered database.

**Enrichment factor (EF):**  $EF = (\text{number of true positives/number of hits in hitlist})/(\text{actives in database/database size})$ .

**False negative hit:** bioactive compound that is not recognized by a model.

**False positive hit:** inactive compound that fits into a model.

**Hit:** a compound that maps the chemical features of a pharmacophore model.

**Target:** pharmacologically relevant protein or nucleic acid which function can be altered by small molecule binding.

**True negative hit:** inactive compound that does not fit into a model.

**True positive hit:** bioactive compound that is correctly recognized by a model

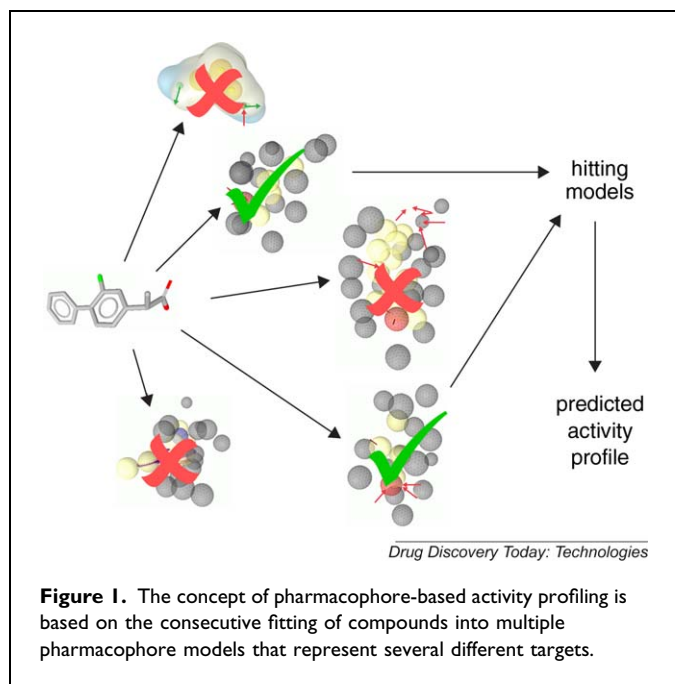
covering different binding sites (e.g. active site vs. allosteric site), different binding modes to the same binding site, restrictive models for cherry-picking and general models for scaffold hopping and antitarget profiling. It is therefore helpful to have a PMC database in which the respective models are categorized and available for selection. Model categorization may be accomplished according to pharmacological target, model purpose or medical indication, to name a few. For example, a model for cytochrome P450 (CYP) 3A4 inhibitors could be categorized using the following pharmacological target ontology: enzymes → EC1.-(oxidoreductases) → monooxygenases → CYP 3A4.

The practical generation of a pharmacophore model set for one target can be set up as described for cyclooxygenase (COX) inhibitors [3]. First, structure-based pharmacophore models are designed using data on protein–ligand complexes

from the Protein Data Bank (PDB) [4] or from proprietary sources. These models are evaluated against a data set of active and inactive ligands or decoys for the respective target, for example, a literature data set, the ChEMBL database [5], the DUD database [6], or the PubChem database [7]. Highly active ligands that are not correctly recognized by this model set may then be used for ligand-based modeling to cover all important compound classes for this target. Fully automated ligand-based pharmacophore model generation using chemically diverse compounds is not recommended, because experts with knowledge about ligands and the protein binding site usually develop models of better quality [8]. The aim of this modeling process is to establish a PMC that correctly identifies a high fraction of active compounds (about 90%). The advantage of using multiple models of sufficient restrictivity, so-called local models, in comparison to employing one more general (global) model is higher specificity, that is, a lower false positive hit rate. To maintain this advantage, it is also important to exclude models with redundant information, that is, models which retrieve the same active compounds as other models. For example, the overall enrichment factor (EF) for a COX PMC of 39 structure-based models was 2.70 on the DUD COX active and decoy data sets. After the removal of redundant models, the 5 remaining pharmacophores had an improved EF of 10.55 [3].

## Evaluation studies

The evaluation of a PMC is crucial before its application for virtual activity profiling. Steindl *et al.* reported virtual screening of 100 antiviral compounds against respective pharmacophore models representing five pharmacologically unrelated antiviral targets [9]. They report 88% correct activity predictions for this data set. In another study, this group evaluated the performance of their PMC for related targets by screening different protease inhibitor data sets against HIV protease inhibitor pharmacophore models [10]. Furthermore, this group compiled models for inhibitors and substrates of several CYP enzymes and evaluated them on the biologically determined CYP activity profile of 17 drugs [11]. Finally, broad activity profiling of PPAR ligands against a PMC consisting of 1537 models for 181 pharmacological targets was performed to determine how well the correct target was predicted [12]. All these evaluation studies aimed at the identification of potential gaps and caveats when using the PMC in a prospective screening. Additionally, they highlighted several unspecific models that would lead to false activity predictions in many cases and should therefore be removed from the PMC. In other cases, prediction gaps were discovered where active ligands were not correctly recognized, pointing out that the PMC needed to be extended by respective models. A summary of these examples is given in Table 1.



**Table 1. Exemplary PMC evaluation studies and their lessons for *in silico* profiling**

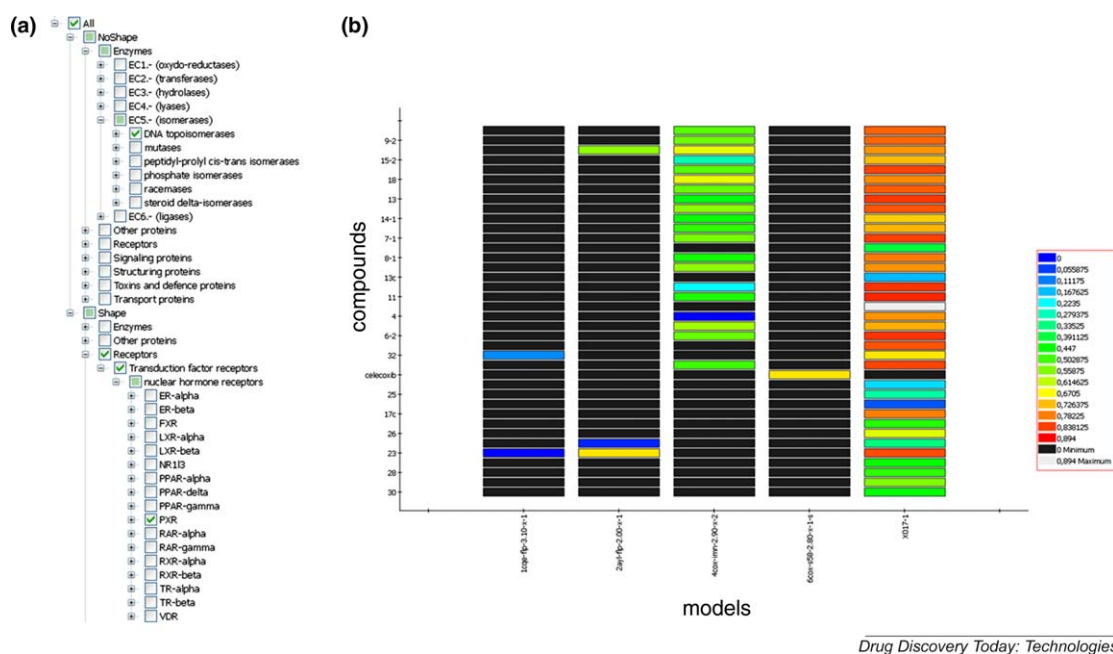
Evaluation study	Focus	Profiling Setup	Results	Ref.
<b>Antiviral compounds</b>	First pharmacophore-based activity profiling application on unrelated targets	100 antiviral ligands screened against 50 pharmacophore models for five unrelated antiviral targets	88% correct predictions, some models for HIV reverse transcriptase turned out to be too global	[9]
<b>HIV protease inhibitors</b>	Discrimination of ligands for related targets	HIV protease inhibitor models used to screen HIV protease inhibitors, inactive compounds, random (virtual) compounds, and other protease inhibitors	Pronounced higher retrieval of HIV protease inhibitors compared to other data sets	[10]
<b>CYP inhibitors</b>	Correct identification of actives for safety profiling	16 models for substrates and inhibitors of five CYPs used to profile 17 drugs with known CYP activity	92% predictions correct or false positive, 8% false negative, mainly CYP3A4	[11]
<b>PPAR inhibitors</b>	Target ranking	1537 models for 181 pharmacological targets used to profile 321 PPAR agonists	For 54% of the 321 PPAR agonists, the PPAR target was ranked 1st or 2nd. Other targets involved in metabolism were also highly ranked.	[12]

### Software solutions

The immense number of models needed for building a PMC applicable to broad activity profiling needs suitable software solutions enabling rapid pharmacophore model building and evaluation. Models may be generated using popular software packages such as DiscoveryStudio (<http://www.accelrys.com>), MOE (<http://www.chemcomp.com>) and Phase (<http://www.schrodinger.com>), or LigandScout (<http://www.inteliland.com>). When working with different software packages, it has to be considered that they handle pharmacophoric features and exclusion volume spheres in different

ways, so that one cannot expect that the same model finds the same hits in all these programs [13]. Although employing different pharmacophore modeling and screening software can therefore lead to a better coverage of chemical space, it is not recommended for pharmacophore-based activity profiling, because PMC evaluation studies are even more difficult to handle in a multi-program environment.

Automated pharmacophoric profiling is readily available in DiscoveryStudio. In the 'Ligand Profiler' protocol, the user can directly select several pharmacophore models for concomitant screening, although without the possibility to add



**Figure 2.** (a) Pharmacophore model classification and selection tree implemented in the Ligand Profiler protocol of DiscoveryStudio. (b) Activity profiling report – the so-called heat map – mapping the models (x-axis) against the compounds (y-axis). The geometric fit values of the compounds into the models are color-coded from low fit (blue) to high fit (red). Non-fitting compounds are displayed in black.

background information for each model. Alternatively, models can be compiled into the user's own Ligand Profiler database, where for each model classification information can be indicated. For the profiling using this Ligand Profiler database, a classification tree is available from which the individual models can be selected (Fig. 2a). Along with DiscoveryStudio, the so-called HypoDB, a proprietary PMC (<http://www.inteligand.com>) can be licensed which consists of over 2500 pharmacophore models representing about 300 pharmacological targets. Although DiscoveryStudio is so far the only software offering automated pharmacophore-based activity profiling and reporting (Fig. 2b), data pipelining software such as PipelinePilot (<http://www.scitegic.com>) or KNIME (<http://www.symyx.com>) facilitate the setup of parallel screening and reporting for practically all virtual screening methods.

### Application fields for pharmacophore-based activity profiling

Although using more than one pharmacophore model for virtual screening has been exemplified before (e.g. [14,15]), the upscaling of this concept to hundreds or thousands of models deserves special attention. Such broad pharmacophore-based activity profiling is a quite recently established approach which required some studies how to deal with such a great amount of prediction data. Since its introduction in 2006, several successful examples with different applications have been reported. The most attractive potential of *in silico* activity profiling in general is to identify multiple pharmacological targets for the screened compounds, which is frequently termed target fishing. This feature can be exploited into several directions.

#### Scenario 1: drug repositioning

Besides the classical drug discovery and development workflow based on virtual screening of chemical libraries, high throughput screening, lead validation and optimization, studies are ongoing that search for new uses of already established drugs. For example, the teratogenic compound thalidomide (Contergan) shows good activity against erythema nodosum leprosum, a complication of leprosy, and is an effective treatment for multiple myeloma and related plasma cell disorders [16]. *In silico* profiling of old drugs can help to reposition such old drugs for new uses [17]. For example, Dubus *et al.* reported a 2D pharmacophoric fingerprints-based similarity search method to identify novel targets for already used drugs [18]. Basis of their predictions was a database of about 650,000 ligands associated with over 2 million biological activities. From a search, bioactivities of the query compound (highest rank) and similar compounds are reported. For each of their query drugs, already known as well as novel targets were reported by their screening platform, thereby offering new ideas for biological experiments.

#### Scenario 2: natural product profiling

Pharmacophore-based activity profiling of natural products offers several possibilities to the research community. On the one hand, the mode of action of ethnopharmacologically used drugs and their single constituents can be predicted to guide pharmacological activity rationalization experiments. On the other hand, novel natural products with interesting chemical scaffolds and unknown bioactivity can be profiled, which may accelerate the discovery of a valuable lead compound for drug development.

One example for natural product-focused activity profiling is the application of a COX inhibitors pharmacophore model collection [3] to constituents of the Thai traditional medicine Prasaplai, which consists of ten plants (mainly spices), camphor and sodium chloride [19]. The analysis of the predictions and a comparison with literature data confirmed COX inhibition as one mode of action for this medicine. A broader activity profiling study has been reported by Ehrman *et al.* [20]. The group built multiple pharmacophore models for COX-1 and -2, p38 MAP kinase  $\alpha$ , Jun kinases 1 and 3 as well as phosphodiesterases 4B and 4D, which are all known targets for anti-inflammatory drugs. Compounds from their in-house Chinese herbal constituent database were profiled against all models, followed by hit ranking using docking. Thereby, several herbs which could exert their effects via one or more than one of these targets were identified. Rollinger *et al.* went a step further and profiled 16 previously isolated main constituents from the aerial parts of *Ruta graveolens* L. against 2208 pharmacophore models covering over 280 unique pharmacological targets from various indications [21]. Selected compounds were tested against acetylcholinesterase, human rhinovirus coat protein, and the cannabinoid receptor 2. 14 out of 18 (77.8%) biologically evaluated predictions were correct, one compound was even confirmed as a multi-target inhibitor. Especially in the natural product field, where a lot of effort is put into the isolation and structure elucidation of compounds and where the amounts of available substance is often limited, *in silico* activity profiling can aid in the discovery of exciting bioactivities [22].

#### Scenario 3: multitarget approach

As mentioned above, many drugs and natural products are already known to act via more than one target. The idea of multi-targeting is that mild interventions with several targets will add up their effects while avoiding toxicity mediated by a total knock out of a single target. Especially pharmacological targets with baseline activity, that can be induced in pathologic conditions, shall not be completely inhibited [23]. It can be favorable to mildly modulate several targets associated with one pharmacological pathway, for example, the arachidonic acid cascade, or from complementary mechanisms involved in a disease, as is often the case for anti-cancer treatment [24]. The pharmacophore-based search for multi-

target compounds is still in its infancy. Apart from the natural product-focused studies described above, no pharmacophoric profiling-based multitarget screening has been reported until now. Current projects in our and other groups promise more reports on multitarget-oriented activity profiling studies in the near future.

#### Scenario 4: safety profiling

The concomitant and early prediction of desired activities and adverse effects of a compound mainly aims at reducing dropout rates in late stages of drug development. In general, *in silico* profiling can guide the selection of lead compounds for drug development programs when several candidates are available. Interactions with so-called antitargets can occur via ion channels, most prominently the hERG potassium channel, G protein-coupled receptors such as the adrenergic  $\alpha_{1A}$  receptor, enzymes like the CYP family, nuclear receptors such as the pregnane X receptor, and others. For many antitarget ligands, pharmacophore models have successfully been established and reviewed recently [25,26]. For example, a PMC consisting of models for CYP 1A2, 2C9, 2C19, 2D6 and 3A4 inhibitors and substrates, respectively, could predict the interactions of 17 drugs and drug candidates with these enzymes with an accuracy of 64% [11]. This success rate may seem very modest. However, antitarget profiling usually requires more general models in order not to miss potentially toxic compounds. In the respective study, 27% of all predictions were false positive ones, meaning that these predictions would have triggered false alarm signals in the profiling. Finally, only 8% of all predictions were false negative ones,

most of them by CYP 3A4 models. Accordingly, for the designated application, this PMC performed sufficiently well.

The amount of data returning from activity profiling can be an issue. There are sometimes 'frequent hitting targets' in the activity profile, especially drug-metabolizing enzymes and transporters, which can indeed bind many chemically diverse compounds. The simplest way to avoid data overflow is to only use models for targets that the user is most interested in. That may be proteins for which biological testing is available in-house or other criteria such as indication areas. Another possibility is to tag or weight all models from the PMC and use this information to interpret the predicted activity profile.

#### Other *in silico* activity profiling approaches

*In silico* bioactivity profiling can be performed using basically any virtual screening method. Not only the interaction with specific targets can be predicted – from a compound's solubility to its bioavailability and distribution in the human body, followed by its biotransformation and excretion, every step can be put into mathematical models and therefore computationally predicted [27]. One of the most prominent and publicly available screening tools is the Similarity Ensemble Approach (SEA) approach [28], available at <http://www.sea.docking.org>. Predictions in SEA are based on ligand similarity, where the query ligand is compared to compounds with known bioactivities from databases. Other reported activity profiling studies used support vector machines [29,30], docking [31–33], structural clustering [34], binding site shape [35], and ligand-based descriptor models [36,37]. The 2D descriptor based program PASS and its success stories have recently been reviewed [38].

**Comparison Summary Table.**

	Drug repositioning	Natural product profiling	Multitarget activity profiling	Safety profiling
	Activity profiling of already known drugs. Drugs can be optimized for another therapeutic application	Activity profiling of natural products	Systematic search for compounds with several targets	Activity profiling using antitarget models
<b>Pros</b>	Advantageous ADMET properties	Expensive or rare natural product material is saved Mode of action rationalization of ethnopharmacologically used drugs enables their use in Western medicine	Compounds with synergistic actions on several targets may enhance therapeutic value and reduce side effects	Fast identification of the most probable toxic compounds Prioritization among several drug candidates facilitated
<b>Cons</b>	Limited to targets which are represented by respective pharmacophore models Patentability issues possible	Natural product databases or already isolated and structurally defined compounds needed as starting points Limited to targets which are represented by respective pharmacophore models	Usually relatively low activity on individual targets	<i>In silico</i> safety profiling cannot replace biological testing
<b>References</b>	[17]	[22,39]	[23]	[40]



Although the compilation of a high quality PMC is a time-intensive endeavor which requires good modeling skills, the so-far published applications from the literature give an insight into the high potential of pharmacophore-based activity profiling, thereby rewarding the initial model building efforts. In comparison to other *in silico* activity profiling technologies, it does not require structural information on the pharmacological targets such as docking-based approaches, because ligand-based pharmacophore models can be employed. Unlike similarity based methods, pharmacophore-based profiling does not depend on already reported structurally similar and active compounds for comparison. Therefore, especially activity predictions for compounds from a non-drug like chemical space, for example, environmental chemicals, may be facilitated. In terms of speed, pharmacophore-based activity profiling lies between the computationally expensive docking approach and the fast 2D similarity search method. One of the most interesting questions for the near future will be the assessment of what advantages a combination of complimentary *in silico* activity profiling techniques could bring. The different *in silico* activity profiling approaches are summarized in the [Comparison Summary Table](#).

## Conclusion

From the featured applications it becomes clear that the described scenarios cannot be strictly divided. The readout of an *in silico* activity profiling run always returns predictions on modes of action, probably via multitarget activity, as well as potential adverse drug reactions or drug–drug interactions, if such models are included in the PMC. The researchers may then decide in which direction to interpret the results. In comparison with some other activity profiling technologies, pharmacophore-based activity profiling offers several advantages. In brief, pharmacophore models are more universal because they are not dependent on the 3D structure of a target and can be applied to scaffold hopping. Additionally, the results can be visually inspected by fitting the compounds into the respective hitting pharmacophore models or directly into the 3D structure of the protein, whenever structure-based models were used. With the availability of commercial software that can handle pharmacophore-based activity profiling and result reporting, much is to be expected from this technology in terms of lead identification and safety profiling.

## Conflict of interest

The author declares no conflict of interest.

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