Pharmacophores and Pharmacophore Searches

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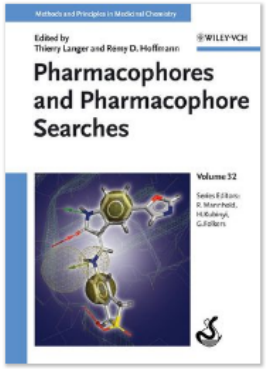

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Thierry Langer (Editor), Remy D. Hoffmann (Editor), Raimund Mannhold (Series Editor), Hugo Kubinyi (Series Editor), Gerd Folkers (Series Editor)

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Description

This handbook is the first to address the practical aspects of this novel method. It provides a complete overview of the field and progresses from general considerations to real life scenarios in drug discovery research.
Starting with an introductory historical overview, the authors move on to discuss ligand-based approaches, including 3D pharmacophores and 4D QSAR, as well as the concept and application of pseudoreceptors. The next section on structure-based approaches includes pharmcophores from ligand-protein complexes, FLIP and 3D protein-ligand binding interactions. The whole is rounded off with a complete section devoted to applications and examples, including modeling of ADME properties.
With its critical evaluation of pharmacophore-based strategies, this book represents a valuable aid for project leaders and decision-makers in the pharmaceutical industry, as well as pharmacologists, and medicinal and chemists.

The Idea

The idea is very straightforward: find and define all locations in space at a certain time of all substituents of a bioactive molecule that contribute to its biological activity. The readout would be a three-dimensional map – with respect to structure – that represents a minimal set of substituents which would adapt to a negative casting mold of the target binding site. By estimating or calculating the electronic and geometric properties of the substituents at their locations you would expand the 3D map to multiple dimensions.

You call it a pharmacophore.

The Idea

After that, theoretically, you would walk through the Periodic Table and create a set of substituents, tied together by an appropriate backbone to fulfill all electronic and steric requirements of the pharmacophore. Finally, you obtain a new chemical entity with good prospects for activity at the target of choice.

But you get more.

A “map” is a tool that relates objects to each other. These relations may be distances as they appear on a roadmap, it may be frequencies or densities on a web exploration map or it may be metabolism–emotion relationships in a brain map. Hence the pharmacophoric map can be used as a filter by matching the property vectors and a library of synthetic and/or virtual ligands, sorting out putative binders.

Camille G. Wermuth

Since the appearance of computer-aided structure–activity studies, the term “pharmacophore” has become one of the most popular words in medicinal chemistry. However, depending on their scientific background and/or traditions, the different medicinal chemistry groups attribute various meanings to this term. Therefore, it appeared necessary to devote a brief paragraph to the definition of the word pharmacophore, and this is followed by a historical perspective and finally by some comments from a medicinal chemistry practitioner.

Definitions

Many authors use the term “pharmacophores” to define functional or structural elements possessing biological activity. This does not correspond to the official definition elaborated by an IUPAC working party and published in 1998: *A pharmacophore is the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response*

Definitions

1. The pharmacophore describes the essential, steric and electronic, function-determining points necessary for an optimal interaction with a relevant pharmacological target.
2. The pharmacophore does not represent a real molecule or a real association of functional groups, but a purely abstract concept that accounts for the common molecular interaction capacities of a group of compounds towards their target structure.
3. Pharmacophores are not specific functional groups (e.g. sulfonamides) or “pieces of molecules” (e.g. dihydropyridines, arylpiperazines).

A pharmacophore can be considered as the highest common denominator of a group of molecules exhibiting a similar pharmacological profile and which are recognized by the same site of the target protein. However, despite the official definition and the remarks made above, many medicinal chemists continue to call pharmacophores some specific functional groups, especially if they appear to be often associated with biological activity

Functional Groups Considered as Pharmacophores: the Privileged Structure Concept

The retrospective analysis of the chemical structures of the various drugs used in medicine led medicinal chemists to identify some **molecular motifs** that are associated with high biological activity more frequently than other structures. Such molecular motifs were called **privileged structures** by **Evans** et al., to represent *substructures that confer activity to two or more different receptors*.
The implication was that the privileged structure provides the scaffold and that the substitutions on it provide the specificity for a particular receptor.
Among the most popular privileged structures, historical representatives are arylethylamines (including indolylethylamines), diphenylmethane derivatives, tricyclic psychotropics and sulfonamides. Dihydropyridines, benzodiazepines, [2, 5], *N*-arylpiperazines, biphenyls and pyridazines [6] are more recent contributions.

A statistical analysis of NMR-derived binding data on 11 protein targets indicates that the biphenyl motif is a preferred substructure for protein binding.

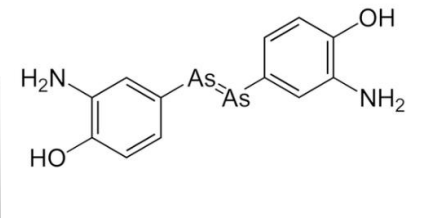
Early Considerations About the Concept of Receptors

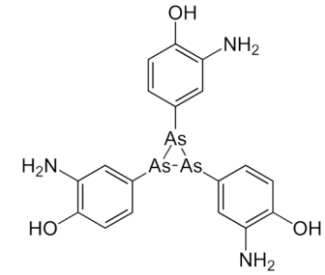
The idea that drugs act upon receptors began with **Langley** in 1878, who introduced the term "*receptive substance*". However, the word "*receptor*" was introduced later, by **Paul Ehrlich**. During the first half of the 20th century, several observations highlighted the critical features associated with the concept of receptor.

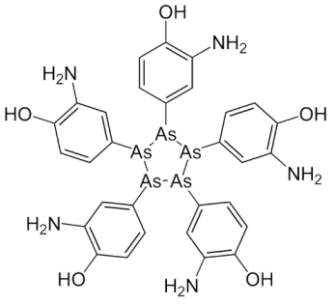
"Three striking characteristics of the actions of drugs indicate very strongly that they are concentrated by cells on small, specific areas known as receptors. These three characteristics are (i) **the high dilution** (often 10–9 M) at which solutions of many drugs retain their potency, (ii) **the high chemical specificity** of drugs, so discriminating that even d- and l-isomers of a substance can have different pharmacological actions, and (iii) **the high biological specificity** of drugs, e.g. adrenaline has a powerful effect on cardiac muscle, but very little on striatal muscle."

Ehrlich's "Magic Bullet"

Selective interaction of a drug molecule with the corresponding receptor was not always accepted. One of the most brilliant demonstrations came from Paul Ehrlich's discovery of salvarsan, which gave rise to the concept of a chemotherapeutic "magic bullet" against specific infectious organisms. Beginning with dyes and later extending his studies to include arsenical compounds, Ehrlich modified the chemical structure of numerous molecules to produce effective drugs against trypanosome and later spirochete infections. Clinical tests confirmed the potential of the drug in treating syphilis and trypanosomiasis. The discovery was announced in 1910. Ehrlich named the drug salvarsan. The German physician, bacteriologist and chemist Paul Ehrlich shared the Nobel Prize in 1908 with Ilya Metchnikoff for their contributions to immunity.





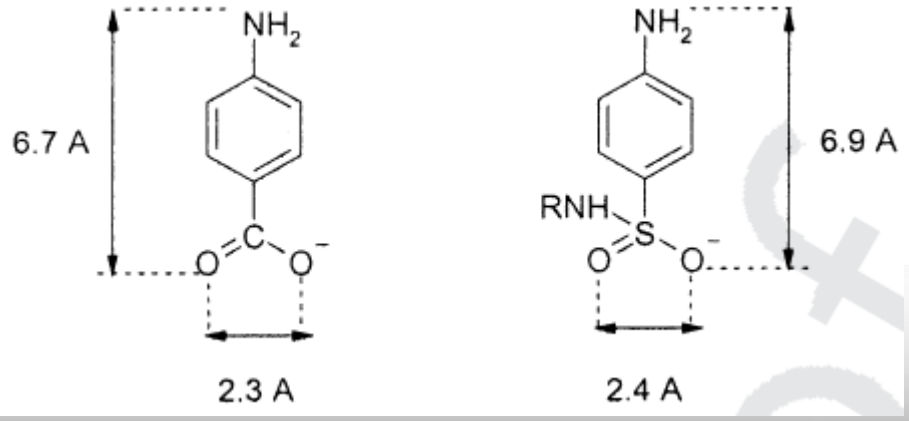


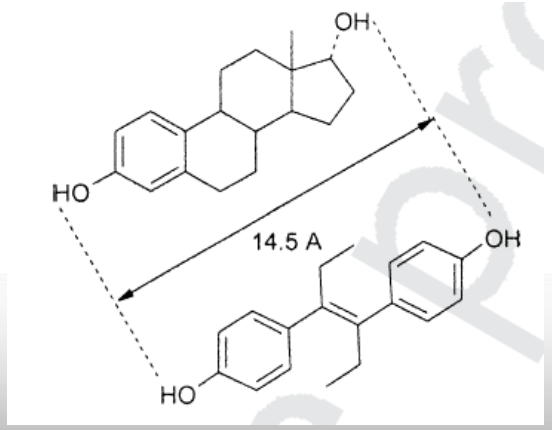
Fischer's "Lock and Key"

Ehrlich's seminal discoveries reinforced the assertion made in 1894 by another brilliant German chemist, Emil Fischer. In a publication dealing with the effect of glucoside conformation on the interaction with enzymes, he wrote: "To illustrate, I would like to say that enzyme and glucoside must fit together like lock and key, in order to have a chemical effect on each other". The image of "**lock and key**" is still used today, even if it suggests a rigid structure of the receptor or enzyme protein. Probably another image, such as "**hand in a glove**", would be more accurate. Effectively, in addition to the steric complementarity, it would account for chirality and receptor flexibility.

Pharmacophores: the Viewpoint of a Medicinal Chemist → Two-dimensional Pharmacophores

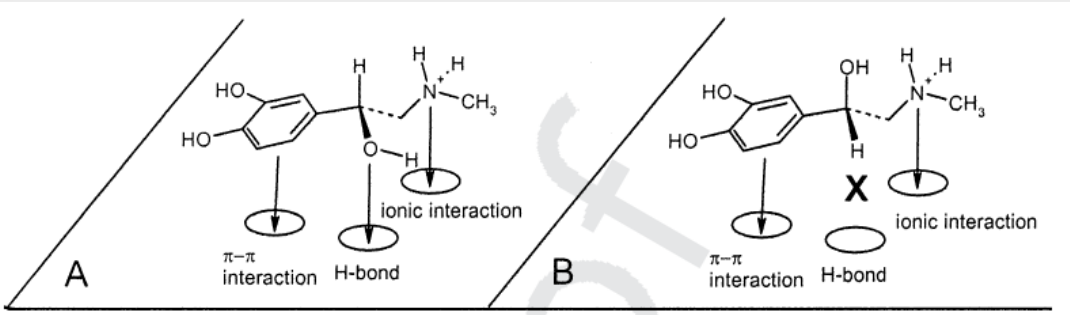
The recognition of the quantitatively almost unmatched ability of ***p*-aminobenzoic acid** (PABA) to oppose the bacteriostatic efficiency of the **sulfonamides** led **Woods** and Fildes to formulate the fundamentals of the theory of metabolite antagonism.
Another early achievemens was the synthesis and the pharmacological evaluation of ***trans*-diethylstilbestrol** as an estrogenic agent showing similarities with **estradiol**. Here again the proposed model was two-dimensional, despite the fact that the non-planar conformation of estradiol was already known.





An Early Three-dimensional Approach: the Three-point Contact Model

When an asymmetric center is present in a compound, it is thought that the substituents on the chiral carbon atom make a three-point contact with the receptor. Such a fit insures a very specific molecular orientation which can only be obtained for one of the two isomers. A three-point fit of this type was first suggested by **Easson and Stedman**, and the corresponding model proposed by **Beckett** in the case of (*R*)-(–)-adrenaline [= (*R*)-(–)-epinephrine].



the natural (*R*)-(–)-epinephrine establishes a threepoint interaction with its receptor (A), the combination of the donor–acceptor interaction, the hydrogen bond and the ionic interaction will be able to generate energies of the order of 12–17 kcal mol–1, which corresponds to binding constants of 10^{-9}–10^{-12}. The less active isomer, (*S*)-(+)-epinephrine, may establish only a two-point contact (B). The loss of the hydrogen bond interaction equals 3 kcal mol–1, hence this isomer should possess an 100-fold lesser affinity. Experience confirms this estimate. If we consider less abstract models, it becomes apparent that the less potent enantiomer also is able to develop three intermolecular bonds to the receptor, provided that it approaches the receptor in a different manner. However, the probability of this alternate binding mode to trigger the same biological response is close to zero

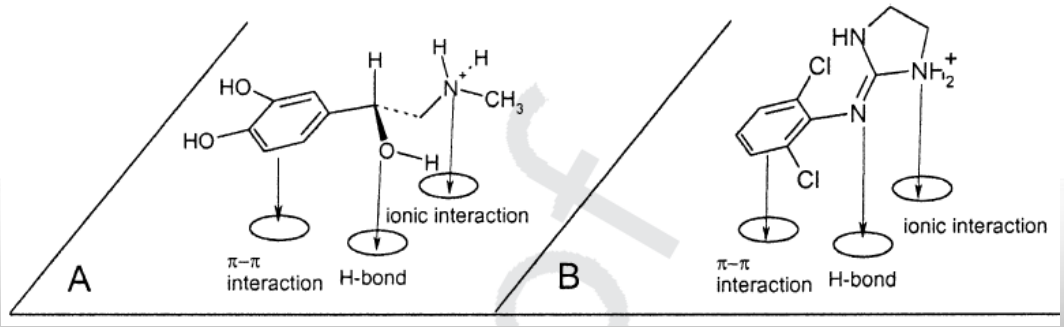
An Early Three-dimensional Approach: the Three-point Contact Model

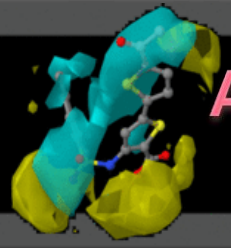
In the early 1970s, it was accepted that the hypotensive activity of clonidine was due to its direct interaction with the central norepinephrine receptor. To trigger the -adrenergic receptor, it was accepted that norepinephrine binds to its receptor by means of three bonds:

- an ionic bond between the protonated amino function and an anion (carboxylate, phosphate) of the receptor active site;
- a hydrogen bond between the secondary alcoholic hydroxyl and a, NH–CO function of the receptor;
- a stacking (or charge transfer?) between the aromatic ring and an electron-deficient ring such as a protonated imidazole of a histidine residue.

In addition, it was known that the phenolic hydroxyls are not essential for activity and that the catiobic head should not be too bulky.

Pullmann et al., in their model of the -adrenergic receptor, found the following critical intramolecular distances: *D*=5.1–5.2 Å from N+ to the center of the aromatic ring and *H*=1.2–1.4 Å for the elevation of the positive charge to the plane of the aromatic ring.



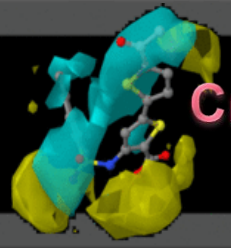


An Early Three-dimensional Approach: the Three-point Contact Model

At first glance, the similarity between clonidine and norepinephrine was not evident; However an NMR structural study of clonidine demonstrated the restricted rotation resulting from dichloro *o*- and *o'*-substitution and imposing a quasi-perpendicular orientation of the imidazolic ring towards the phenyl ring. As a result, clonidine can yield the same kind of interactions as norepinephrine.

Taken together, the examples shown above illustrate typically some pre-computer attempts to elucidate pharmacophoric patterns usable as guides for the design of new drugs.

They prepared the minds for **Garland Marshall**'s seminal publications on computer-aided pharmacophore identification and all the derived applications.



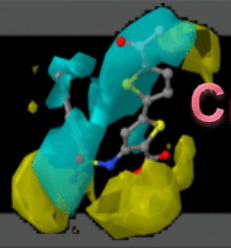
Criteria for a Satisfactory Pharmacophore Model

It has to highlight the functional groups involved in the interaction with the target, the nature of the non-covalent bonding and the different intercharge distances.

The model also has to show some *predictive power* and lead to the design of new, more potent compounds or, even better, of totally novel chemical structures, not evidently deriving from the translation of structural elements from one active series into the other.

An interesting aspect of pharmacophore-based analogue design is referred to as **scaffold hopping**.

It consists in the design of functional analogues by searching within large virtual compound libraries of isofunctional structures, but based on a different scaffold. The objective is to escape from a patented chemical class in identifying molecules in which the central scaffold is changed but the essential function-determining points are preserved and form the basis of a relevant pharmacophore



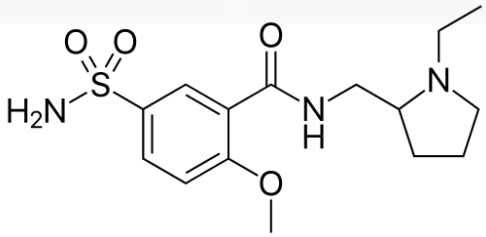
Criteria for a Satisfactory Pharmacophore Model

The second criterion for a valid pharmacophore model is that it should discriminate stereoisomers. Stereospecificity is one of the principal attributes of pharmacological receptors and a perfect stereochemical complementarity between the ligand and the binding-site protein is an essential criterion for high affinity and selectivity. A convincing example of enantiomeric discrimination was observed for GABA-A receptor antagonists.

In a similar manner, the ideal model should distinguish between agonists and antagonists. This is relatively easy for the specific category of antagonists which, according to Arins et al.' theory , derive from the agonists simply through the addition of some supplementary aromatic rings which play the role of additional binding sites (e.g. the passage from muscarinic agonists to muscarinic antagonists or from GABA agonists to GABA antagonists). The discrimination between the two categories becomes less evident when the passage from agonist to antagonist relies on relatively subtle changes such as one observes for glutamate, oxotremorine and benzodiazepine antagonists

Criteria for a Satisfactory Pharmacophore Model

Sometimes a good pharmacophore model can *explain* apparently *paradoxical observations*, e.g. the unexpected affinity reversal found in *R*- and *S*-enantiomers of the sulpiride series on changing *N*-ethyl to *N*-benzyl derivatives.



Finally, it has to account for the *lack of activity* of certain analogues of the active structures. The knowledge of structural or electronic parameters leading to poorly active or inactive compounds is a cost-lowering factor that allows the number of compounds to be synthesized to be reduced.

Combination of Pharmacophores

Some highly specific mono-target drugs have clearly proven the usefulness of mono-target medicine. Examples are phosphodiesterase 5 inhibitors such as sildenafil, the -1a antagonist drugs such as tamsulosine, selective COX-2 inhibitors such as celecoxib and kinase-specific anticancer drugs such as imatinib.

However, in addition to one-target drugs, clinicians are more and more convinced that modulating a multiplicity of targets can be an asset in treating a range of disorders. An extreme example of a multi-target drug is clozapine, which exhibits nanomolar affinities for more than a dozen different receptors.

Combination of Pharmacophores

an increasing number of publications reflect an awakening of interest in the rational design of multiple ligands and may suggest an ongoing re-evaluation of the “one disease, one drug” paradigm which has dominated thinking in the pharmaceutical industry for the last few decades.
Although there is little chance of switching back to the animal-centric approach of the past, it is now widely recognized that high specificity for a single target may not deliver the required efficacy versus side-effect profile and, in many cases, a balanced activity at several targets may produce a superior effect.
In a recent paper, entitled “From magic bullets to designed multiple ligands”, Kay et al. discuss the opportunity and the advantages of ligands acting on two (or more) specific targets, such *intentionally* designed multiple ligands (DM ligands) being opposed to *serendipitous* multiple ligands.

It is highly probable that computer-driven combinations of two pharmacophores can lead to the design of new active entities combining in one molecule the critical structural elements of two partners.

Pharmacophore Model Generation Software

Often, all alignment-based methods and molecular field and potential calculations are classified as pharmacophore perception techniques.

referring mainly to one specific type of perception, namely three-dimensional feature-based pharmacophore models represented by geometry or location constraints, qualitative or quantitative. An extrapolation of the pharmacophore approach to a set of multi-dimensional descriptors (pharmacophore fingerprints) has been developed mostly for library design and focusing purposes.

Various ways of perceiving pharmacophores have been explored, known issues with pharmacophore modeling have been addressed in one way or another and several computer-based applications with a pharmacophore focus have been created since the 19803. Many of these programs are not intensively used today: ALADDIN, DANTE, APOLLO, RAPID, SCREEN and its PMapper from ChemAxon and ChemX fingerprints from Chemical Design (now Accelrys).

Molecular Alignments

the terms molecular alignment and superposition and pharmacophore elucidation are often used interchangeably, it is probably more accurate to differentiate alignment as providing a prerequisite to pharmacophore development.
Conversely, some alignment methods require a pharmacophore as a starting point.

Of course, molecular alignment is not limited to just providing a basis for pharmacophore elucidation; it can also be used to derive 3D-QSAR models that potentially can estimate binding affinities, in addition to indirectly providing insight into the spatial and chemical nature of the receptor–ligand interaction of the putative receptor.

Essentially, an alignment endeavors to produce a set of plausible relative superpositions of different ligands, hopefully approximating their putative binding geometry.

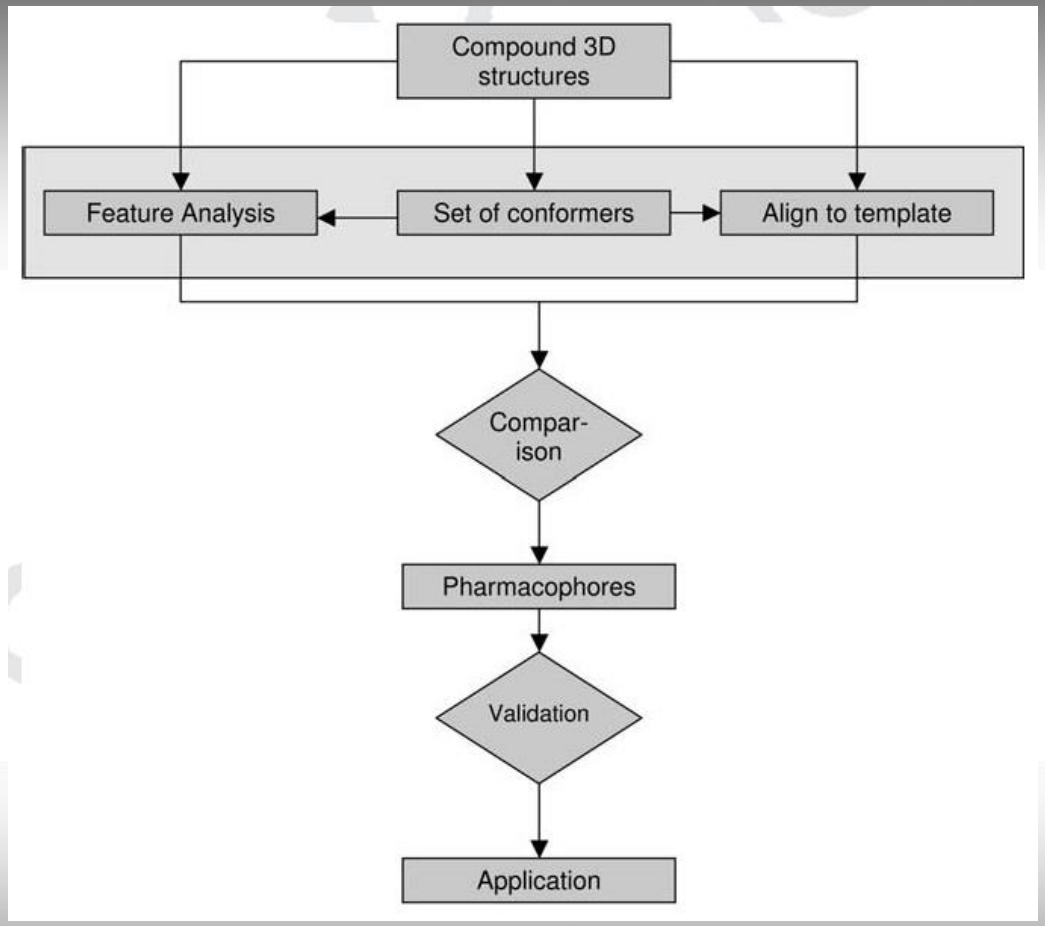
Scoring and Optimization

All alignment methods require some quantitative measure or fitness function, to assess the degree of overlap between the ligands being aligned and to monitor the progression of that optimization. This is most often manifested as a molecular similarity score or alignment index.

Typically in point-based algorithms, the optimization process endeavors to reduce the root-mean-square (RMS) deviation of the distances between the points or cliques by least-squares fitting. However, interesting variations have been developed including the use of distance matrices to represent any given conformation of a ligand [30]. Simulated annealing is used to optimize the fitness function, which is a quantification of the sum of the elements of the difference distance matrix created by calculating the magnitude of the difference for all corresponding elements of two matrices.

Another optimization method, related to the least-squares fitting used in point-based algorithms, is the directed tweak method. This is a torsional space optimizer, in which the rotatable bonds of the ligands are adjusted at search time to produce a conformation which matches the 3D query as closely as possible. As directed tweak involves the use of analytical derivatives, it is very fast and allows for an RMS fit to consider ligand flexibility

Pharmacophore Modeling



Compound Structures and Conformations

The generation of the correct compound structures is a critical step in which different components such as atomic valences, correct bond orders and properly defined aromaticity have to be considered carefully. In addition, the correct stereochemistry flags need to be added for a correct treatment of stereochemistry.

Most of the current pharmacophore generation packages include compound builders, but users can also import them from external sources using common file formats, for example SMILES, MOL, SD or MOL2.

Representation of Interactions in the Pharmacophore Models

The representation of pharmacophores varies from one package to another and includes the nature of the pharmacophore points (fragments, chemical features) and the geometric constraints connecting these points (distances, torsions, three-dimensional coordinate location constraints).

The interpretation of the chemical structures of the molecules can be done at two levels:

1. Substructural, where molecules can be decomposed into different fragments, each fragment carrying certain specifications (e.g. basic nitrogen or aromatic ring).
2. Functional, where an abstraction of the structure is made such that each molecular fragment of the compounds is expressed by the general property it carries. In the current stage, the properties mapped on the fragments are chemical properties, e.g. hydrophobic or ionic interactions or hydrogen bonding features. The characterization of the chemical properties of compounds requires these functions to be accessible for the interaction with the binding partner (receptor, enzyme or nucleic acid), so in case the bioactive conformation of the ligand is not known, a conformational expansion analysis is a necessary step in order to identify a conformation which makes those functions available for interaction with the macromolecular target.

Conformational Expansion

This is probably the most critical step, since the goal here is not only to have the most representative coverage of the conformational space of a molecule, but also to have either the bioactive conformation as part of the set of generated conformations or at least a cluster of conformations that are close enough to this the bioactive conformation. Here we divide the methods that can be used for this purpose roughly into four categories: systematic search in the torsional space, optionally followed by clustering, stochastic methods. The resulting set of conformations can be further optimized using minimization with or without solvent.

Marshall et al. described the so-called Active Analog Approach, in which the conformational space of flexible molecules is constrained to the geometry of a reference molecule (generally active and as rigid as possible). Pharmacophore models are then derived from the set of resulting alignments. This approach has been successfully used since the mid-1980s and still forms the basis of many existing automated pharmacophore modeling techniques.

Comparison

This step constitutes the pharmacophore generation itself and represents the major focus of this chapter. The majority of pharmacophore generation packages generate qualitative pharmacophores that do not consider the activity of the molecules (potency), so in general equipotent molecules have to be used.

Most of these methods are based on minimizing the RMS superposition error between conformations of various compounds while trying to increase the three-dimensional overlay of pharmacophores. The result is generally multiple pharmacophore solutions, ranked according to different metrics depending on the package used.

Pharmacophores, Validation and Usage

After performing pharmacophore analysis on a set of compounds, typically the user will have to select the model(s) with biological and/or statistical relevance, often from multiple possible solutions and use for further research purposes.
The validation of the pharmacophore models is therefore a critical aspect of the pharmacophore generation process.
In a nutshell, these validation methods can be ordered into three categories:

1. Statistical significance analysis, randomization tests.
2. Enrichment based methods. These focus on recovering active molecules from a test database in which a small number of known actives have been hidden in a large database of randomly selected compounds. Database mining and the utilization of receiver operating characteristic (ROC) curves [43] can be included in this category.
3. Biological testing of a selection of compounds.

The main utility of pharmacophores is their use as screening tools. Many examples in the literature show their successful usage in finding new scaffolds

Automated Pharmacophore Generation Methods

In order to have an objective view of the different available pharmacophore perception software tools, we chose to analyze these using the following criteria whenever possible:

1. Compound builder: is there a molecular builder? Which file formats are supported?
2. Stereochemistry: how is the stereochemistry of molecules handled in the program?
3. 3D conformations: does the program contain conformer generation methods?
4. Pharmacophore generation engine: which type of pharmacophore perception engine is implemented in the program?
5. Fitness function: how are the pharmacophores evaluated?
6. Alignment method: does the program require pre-alignment of molecules? On what basis are the molecules aligned together?
7. Pharmacophore definition: description of the type of pharmacophore locations and associated functions. Can other descriptors be added to the pharmacophore alignment?
8. Database searching: is a database search engine implemented in the program? Which types of database searches are possible, e.g. substructure, pharmacophore,
9. shape, exclusion?
10. Scoring of hits: Can database search hits be ranked?