

LigandScout User Manual

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Chapter 1. Introduction to LigandScout

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LigandScout is a powerful structure- and ligand-based pharmacophore model generator based on sophisticated algorithms for performing alignments and interpreting and customizing ligand-macromolecule interactions.

LigandScout extracts and interprets ligands and their macromolecular environment from PDB files and automatically creates and visualizes [advanced 3D pharmacophore models](#) supporting multiple features per heavy atom to broaden the scope of a single model. A wide range of powerful editing tools ([2D View](#) and [3D View](#)) lets you generate customized, highly specific pharmacophore models within a few seconds. [Excluded volume recognition](#) and the resulting [excluded volume coats](#) drastically increase selectivity by considering sterical characteristics of the binding site. Moreover, LigandScout supports the generation of [pharmacophore models based on molecules injected into the binding site](#) and supplies a [Library View](#) for the investigation of docking poses and viewing results of virtual screening in the active site.

Analysis of the conformational space of ligands, clustering and alignment methods help to search for common feature arrangements. This is especially useful when binding site interactions are not known. Reaching a consensus pattern from a set of ligands binding to one particular macromolecule is a further step in drug development. Rapid and accurate in-silico screening of compound libraries is integrated into LigandScout along with tools to analyze the performance of the models.

This manual documents all LigandScout features and gives workflow guidelines for the [generation of structure-based pharmacophores](#), [ligand-based pharmacophores](#), [virtual screening of molecule libraries](#) and [pharmacophore preparation for external virtual screening applications](#).

Next steps:

1. [What's New in LigandScout 3.0](#)
2. [Installing and Starting LigandScout](#)
3. [Visual Semantics](#)
4. [Using LigandScout: The 3D Graphical User Interface](#)

1.1. What's New in LigandScout 3.0?

New features introduced to LigandScout 3.0:

1. [Ligand-Based Modeling](#)
2. [Virtual Screening](#)
3. [Conformation Generation](#)
4. [Conformational Clustering](#)
5. [Tautomer Generation](#)
6. [Molecule Structure Optimization by MMF94 Force Fields](#)
7. [Fast Calculation of Tasks through the Network](#) to increase Execution Performance

1.1.1. Hot Topics

Tautomers

[Tautomeric rearrangements](#) of a molecule lead to distinct equilibrated structural states of the same chemical compound and thus have an influence on nearly all aspects of computer-based chemical data processing. Especially for structure-based pharmacophore modeling of ligand-protein complexes, tautomeric rearrangements are crucial for the presence or absence of possible H-bonding interactions due to changing H-donor/H-acceptor properties. LigandScout provides a rule-based ligand-side tautomer enumeration and ranking procedure that considers both geometrical constraints imposed by the conformation of the bound ligand as well as intra- and inter-molecular energetic contributions.

Force Fields

LigandScout provides a full fledged, test-suite conforming MMFF94 implementation for the energy calculation and geometry optimization of molecular structures. You can calculate the strain energy of compounds in a database, energy-minimize the structure of free molecules or optimize ligands within a binding pocket. The [parameters of the MMFF94 force field](#) can be customized. For instance, you can define which components of the force field have to be considered and specify parameters for several interactions (e.g. distance cutoffs for Van der Waals and electrostatic interactions). During a structure optimization process, SMARTS pattern defined parts of the ligand or molecular environment can be kept fixed.

Conformation Analysis

The “right” ligand conformation is a prerequisite for an energetically favorable interaction with the target receptor. Thus, consideration of multiple ligand conformations can increase significantly the quality and reliability of derived pharmacophore models. LigandScout enables the creation of ligand-based phar-

macophores from single and multiple ligand conformations. Conformations are generated by OMEGA which can be [customized](#) freely to yield optimal results.

Clustering

One of the crucial parts of ligand-based pharmacophore creation is the choice of ligands for the Training-Set and Test-Set. LigandScout offers clustering methods to assist your decision. This functionality groups ligands according to their similarity to each other using [distance calculation methods](#).

1.2. Installing and Starting LigandScout

The current version of LigandScout is available via Internet download from [Inte:Ligand](http://www.inteligand.com/ligandscout/download.shtml) [<http://www.inteligand.com/ligandscout/download.shtml>]. The LigandScout setup assistant will guide you through the installation process. Follow the instructions of the setup assistant to finish the installation; LigandScout is now ready to use.



Figure 1.1. LigandScout installer

Click on the LigandScout icon (at the location selected during the installation process) to start the application.

Upon the startup of LigandScout you will be prompted for the serial number, which was shipped together with your LigandScout program package. You will then need to activate your copy of LigandScout, which requires an established Internet connection. This activation procedure needs to be executed only once. You may use your copy of LigandScout at your convenience during the entire license period.

Please see [Section 2.3, “Local and Shared Repository”](#), if you would like to install a data repository.

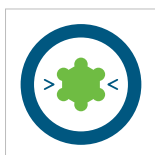
1.3. Visual Semantics

The overview below demonstrates the basic concept for the icons and symbols in LigandScout. It provides quick recognition for the meaning of the program controls.



Chemical feature

Multiple chemical features form a pharmacophore and are responsible for steric and functional chemical interactions between macromolecules and ligands. Chemical features always refer to the ligand side, but take the macromolecule geometry into account in many cases (e.g. hydrogen bond vectors, where the vector target points to the macromolecule counterpart).



Active Site

Represents the binding pocket in the macromolecule. It consists of a core (ligand molecule), an environment (surrounding residues of binding pocket) and potential chemical features.



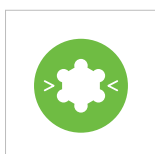
Core

The ligand molecule without the surrounding standard residues (amino acids or nucleic acids).



Environment

The environment includes all macromolecule components (amino acids or nucleic acids) that LigandScout considers to be relevant for pharmacophore generation and thus biological interaction. A structure-based pharmacophore is always created based on interactions between core and environment. Molecules, such as co-factors, waters and ions can be moved between the two sides allowing different views of interactions to be reflected for pharmacophore generation. A ligand-based pharmacophore is generated considering only features of a ligand (set).



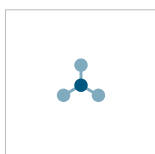
Pharmacophore

A pharmacophore is the set of chemical and steric interactions between a ligand and macromolecule that are relevant to trigger biological response.

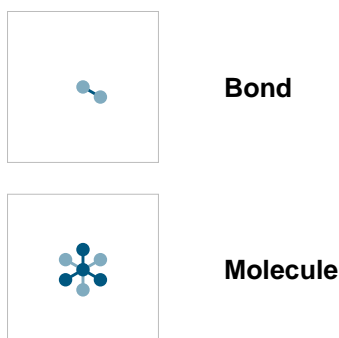


Excluded Volume Spheres

Excluded volumes indicate areas which are sterically forbidden for the ligand (e.g. because of the presence of the protein backbone or a lipophilic interaction).



Atom



1.4. LigandScout Graphical User Interface

LigandScout uses an intuitive graphical user interface (GUI) for sophisticated pharmacophore generation and data analysis. It provides five perspectives, which have different functionalities:

1. **Structure-Based Modeling Perspective**
Analysis of ligand-macromolecule interactions. Viewing and calculating properties of molecule libraries in the PDB View. Managing binding sites in the Bookmark View.
2. **Ligand-Based Modeling Perspective**
Analysis of common interaction patterns of a set of ligands.
3. **Alignment Perspective**
Aligning of pharmacophore model(s) and/or molecule(s).
4. **Screening Perspective**
Screening molecule libraries using pharmacophores.

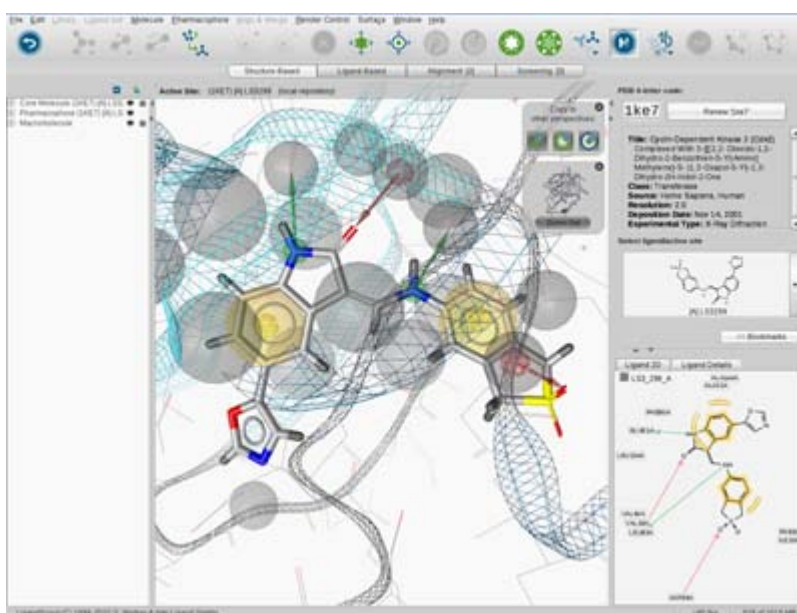


Figure 1.2. Graphical user interface

The [Figure 1.2, “Graphical user interface”](#) shows the Structure-Based Modeling Perspective of LigandScout. LigandScout contains a menu, toolbar, five perspectives and control panels (in this case, the PDB Panel is shown). Furthermore, LigandScout provides modules such as interactive 2D and 3D visualization areas, *Library View* and *Hierarchy View*, which help to orientate and navigate through your data. The visibility of the control panels, *2D View* and *Hierarchy View* can be toggled in the *Window* menu. LigandScout provides universal *Undo* and *Redo* functionality that lets you go back and forth in history.

For more information about the five perspectives please see [Chapter 3, LigandScout Perspectives](#).

Chapter 2. File Handling, Data Management and Networking

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Investigation of molecular information produces a lot of data - especially if you work on diverse projects, e.g. data from PDB complexes, molecule and/or pharmacophore alignments, molecule libraries, screening results, etc. LigandScout offers various ways to keep an overview and organize your data.

1. [Importing and Exporting Files](#)
2. [Retrieving PDB Complexes Online](#)
3. [Local and Shared Repository](#)
4. [Sessions](#)

In terms of data management it is important to efficiently store your data - especially when dealing with large data amounts. On the other hand it is equally important to efficiently process your data. For instance, a single computer running a single task that screens a multi-conformational molecule database can be very time-consuming. One way to deal with this situation is to make use of multi-core processors. Another way to reduce the overall execution time is to setup a network of remote peers, each one performing a piece of work. LigandScout does support this kind of network execution. Please, refer to [Section 2.5, "Networking"](#) which discusses this topic in detail.

2.1. Importing and Exporting Files

LigandScout supports commonly used file formats as well as its own file formats to exchange information. As the main focus of LigandScout is drug development and pharmacophore modeling, all file formats either deal with molecular data, pharmacophore models, or display-related information.

2.1.1. Importing Files

The *File* menu provides two possibilities to import data: the *Open* command (CTRL+O) and the *Insert* command (CTRL+I). Both trigger the display of the file dialog that facilitates the import of files that are being loaded into the current view, whereas the former clears the view first.



Figure 2.1. Open file dialog

The supported import file formats can be differentiated into LigandScout, molecule and other file formats. The following table gives an overview of the file formats including name and file extension list.

File Format	Name	Extension(s)
LigandScout	Library in Local Database	.ldb
	Ligand Set Description	.lsd
	Pharmaceutical Markup Language	.pml
	Compressed Pharmaceutical Markup Language	.pmz
	Session	.ses
Molecule	MDL Molfile	.mol
	Tripos Sybyl MOL2 Molecule	.mol2
	MDL Structure-Data	.sd .sdf
	GZip Compressed MDL Structure-Data	.sd.gz .sdf.gz
	Zip Compressed MDL Structure-Data	.sd.zip .sdf.zip
	Daylight Chemical SMILES String	.smi
Other	Protein Data Bank (PDB)	.pdb .ent
	GZip Compressed Protein Data Bank (PDB)	.pdb.gz .ent.gz
	Zip Compressed Protein Data Bank (PDB)	.pdb.zip .ent.zip
	Molecular Discovery GRID	.kont

Table 2.1. Supported import file formats

The PDB file format provides 3D information about the macromolecule from X-ray or NMR sources. This data plays an important role in deriving a pharmacophore model representing the bound state of a ligand inside a protein binding pocket. The Molfile characterizes a single molecular structure whereas SD(F) and MOL2 files can store more than one molecule (molecule library). In LigandScout, a single molecule

can be used to create a pharmacophore model or to align to other molecules/pharmacophores. The latter allows LigandScout to import compound collections. For instance investigations of these ligands with respect to their protein environment, and also for generating pharmacophores based on docking poses. Moreover, LigandScout can filter molecules from a molecule library that fulfill a desired pharmacophoric pattern. Compared to the other file formats, SMILES files represent only the 2D depiction of the molecules. The [2D View](#) of LigandScout uses the 2D information of the ligand to visualize the derived pharmacophoric features.

The PML file format can be stored and opened with different combinations of content elements, for instance a pharmacophore of a ligand-protein complex or alignments of molecules. The PMZ format contains the compressed PML data. The LSD file format is used in the [Ligand-Based Modeling Perspective](#) to generate pharmacophores from a set of ligands. The LDB file format are used to store/load molecule libraries. This can be useful for investigations of compounds with respect to their protein environment, and also for generating pharmacophores based on docking poses. For more information on advanced export settings, see also [the section called “Advanced Export Settings”](#).

The GRID Map file format can be imported to LigandScout to analyze energetically favorable regions in the binding pocket of a protein. For more information about this topic, please see [the section called “GRID Maps Visualization”](#).

2.1.2. Exporting Files

LigandScout supports data export in several chemical and graphical file formats. Use the command (CTRL+SHIFT+S) in the menu *File > Save as File* which enables you to save your macromolecule, ligand modifications, and pharmacophore models. A session can be stored in the menu *File > Sessions > Save as File*. Depending on the selected file format and content, the *Save File* dialog provides [advanced export settings](#).

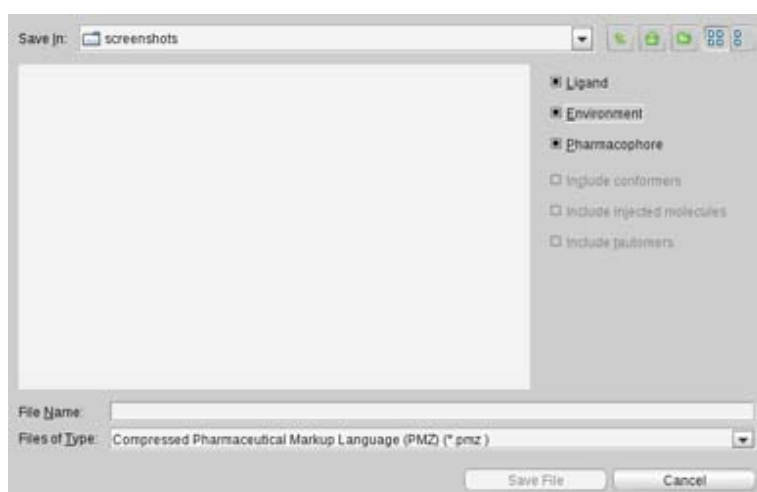


Figure 2.2. Save file dialog

The following table gives you a summary of the supported export file formats together with which data of your work that can be exported in a particular format. Each format facilitates the export of certain

information: molecules (M), ligand environment (E), pharmacophore models (P), and the current content of either 3D View or 2D View (V).

File Format	Name	Extension(s)	M	E	P	V
LigandScout	Compressed Pharmaceutical Markup Language	.pmz	x	x	x	
	Pharmaceutical Markup Language	.pml	x	x	x	
	Ligand Set Description	.lsd	x		x	
	Library in Local Database	.ldb	x			
	Session	.ses	x	x	x	
Molecule	Daylight Chemical Smiles String	.smi	x			
	MDL Structure-Data	.sd .sdf	x			
	GZip Compressed MDL Structure-Data	.sd.gz .sdf.gz	x			
	Zip Compressed MDL Structure-Data	.sd.zip .sdf.zip	x			
	Maestro Molecule	.mae	x			
	MDL Molfile	.mol	x			
	Tripos Sybyl MOL2 Molecule	.mol2	x			
Pharmacophore	Catalyst Hypoedit Script	.hypoedit			x	
	Fred 2 Constraint Map	.fred2map			x	
	Phase Files	.xyz .xvol .def .mae			x	
	CCG MOE Format	.ph4			x	
Image	Encapsulated PostScript	.eps				x
	Graphics Interchange Format	.gif				x
	Joint Photographers Expert Group Format	.jpg				x
	Macromedia Flash File Format	.swf				x
	POV-Ray Scene Description	.pov				x
	Portable Document Format	.pdf				x
	Portable Network Graphics Format	.png				x
	PostScript	.ps				x
	RAW Image	.raw				x
	Scalable Vector Graphics	.svg				x
	GZip Compressed Scalable Vector Graphics	.svgz				x
	UNIX Portable PixMap	.ppm				x
	Windows Enhanced Metafile	.emf				x
Other	Protein Data Bank (PDB)	.pdb .ent	x	x		
	GZip Compressed Protein Data Bank (PDB)	.pdb.gz .ent.gz	x	x		
	Zip Compressed Protein Data Bank (PDB)	.pdb.zip .ent.zip	x	x		

Table 2.2. Supported export file formats

Advanced Export Settings

LigandScout has its own file format, called PML. The advantage of this format is that the user can select the available export content individually or in combination (ligand, environment, pharmacophore, conformers, injected molecules, and tautomers).

LigandScout offers you the possibility to export LigandScout's pharmacophore models so that you can use them in other external programs like Catalyst, MOE and Phase (see [Section 8.2, "Preparing a Pharmacophore for Virtual Screening with External Applications"](#)).

You can save your content as PDB or ENT file format if you want to export the ligand, environment and include hydrogen information. The SMILES string of molecules can also be exported as SMI files. You can include in the files the canonical order of ligand atoms and/or stereo chemical properties. Single molecules can be stored in mol files, as 2D or 3D data. Additionally, stereo-chemical properties can be saved optionally.

The storage of a molecule library in LDB, MOL2 and SD(F) files can be customized. You can export a *Complete library* or a part of it. A subset of a library can be chosen by [filtering your library \[33\]](#) according to a property (e.g. by an occurring name), marking molecules or by visible molecules only. If you want to export the properties of your library table, choose the *Write properties* option. The MOL2 and SD(F) file formats offer you the possibility to *Write all conformations* or *Write 2D/3D coordinates* of the (selected) molecules in the library.

If you want to export currently displayed content as an image file (e.g. gif, svg, etc.) you can choose between the 2D or 3D View. The 2D content can be saved as it is by the *Keep current layout* or you can automatically store it as *horizontal/vertical layout*. After a screening run, you can export the *ROC plot* as image. The image size can be set in both 2D and 3D.

2.2. Retrieving PDB Complexes Online

LigandScout provides the possibility to directly import PDB data from web sources specified in the [Preferences/PDB & Proxy Settings](#). Just type a valid PDB code into the *PDB 4-letter code* field in the control panel of the Structure-Based Modeling Perspective. If the data is available in either the Local Repository or Shared Repository ([Local and Shared Repository](#)) then it is loaded immediately and the button will be labeled *Renew 'PDB code'*. If the PDB complex was not found in the repositories the button will be labeled *Download 'PDB code'*. Anyway, by clicking the button the PDB complex is loaded from the web source, automatically interpreted, stored in the Local Repository, and displayed in LigandScout.

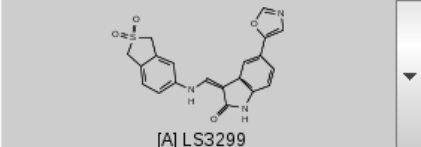
PDB 4-letter code:

1ke7

Title: Cyclin-Dependent Kinase 2 (Cdk2)
 Complexed With 3-[[[2,2-Dioxido-1,3-Dihydro-2-Benzo[thien-5-Yl]Amino]Methylene]-5-(1,3-Oxazol-5-Yl)-1,3-Dihydro-2H-Indol-2-One

Class: Transferase
Source: Homo Sapiens, Human
Resolution: 2.0
Deposition Date: Nov 14, 2001
Experimental Type: X-Ray Diffraction

Select ligand/active site



[A] LS3299

Figure 2.3. PDB panel

LigandScout connects to the PDB servers listed in [Preferences/PDB & Proxy Settings](#) according to their appearance. The listing of the servers from top to bottom is the order in which the servers are contacted until a valid PDB structure is found and downloaded correctly or all listed servers were contacted. After a successful download LigandScout activates the PDB interpretation algorithm, shows the complex in the [Macromolecule View](#), and stores a copy in the Local Repository automatically for faster access if this code should be entered again sometime later. Please see [Local and Shared Repository](#) for detailed information.

If you experience problems connecting to the PDB server(s), please see the [PDB & Proxy Settings](#) in the Preferences/Program & Network Settings for more information.

2.3. Local and Shared Repository

LigandScout uses a “two-level” file storing strategy, the Shared Repository (or Central Repository) and the Local Repository. If a PDB code is entered in the *PDB 4-letter code* field of the [PDB Panel](#), LigandScout tries to find the associated files in the first level Local Repository. If unsuccessful, LigandScout will check the Shared Repository which represents the second level. The Shared Repository is usually installed at a central location and every licensee can take advantage of it by specifying the correct path to this repository in [Preferences/Program & Network Settings](#). As the second level repository is intended to be shared among all licensed users, it is not modifiable using LigandScout (the only exception is if a new repository is installed to be the new Shared Repository). Hence, there is the first level repository which contains all of the changes that were performed on a structure locally (if the user decides to save them). Also, recently downloaded and interpreted PDB structures will be stored there for faster retrieval later on.

2.3.1. Handling the Repository

Save to Repository

You can store changes to your current active site to the Local Repository with *File > Save to Repository* (CTRL+S). If you do not use this command and you load new data or quit LigandScout, a *Save changes* dialog reminds you to store or discard the modified data.

Importing a Repository

The *Import Repository* command (CTRL+ALT+SHIFT+I) in the *File > Repository* menu allows you to install a PDB repository.

Exporting a Repository

The *Export Repository* command (CTRL+ALT+SHIFT+E) in the *File > Repository* menu enables the export of your PDB data storage. To allow sharing your Local and Shared Repository you can choose to store it as a single compressed archive. Choose *Local Repository* for your local storage. If the repository is located on a server and is to be shared with other users, select *Shared Repository*. Make sure that you have writing privilege for this location. The path to your *Shared Repository* is specified in the [Preferences/Program & Network Settings](#) whereas the *Local Repository* will be managed by LigandScout.

Clearing the Local Repository

The *Clear Local Repository* command (CTRL+ALT+SHIFT+C) in the *File > Repository* menu erases the local PDB data storage. All changes and modifications will be lost if the Local Repository is cleared.

2.4. Sessions

Quite similar to the PDB data storage in the Local and Shared Repository LigandScout transparently stores information concerning the Local Repository, [Alignment Perspective](#), [Bookmark View](#), [Ligand-Based Modeling Perspective](#) and [Screening Perspective](#). To be able to manage these data, especially when working on different projects with different data, it seems to be useful to backup your current workspace as an individual session. Then you can load a different session or proceed modeling without altering the stored session.

2.4.1. Handling Sessions

Store current session

The current session can be stored in a SES file or directly in LigandScout. Choose in the menu bar *File > Sessions > Save Session as File* or alternatively, *File > Sessions > Save Current Session* to save your current data in a session by specifying a name. The latter stores the data in the `.inteligand-data` directory.

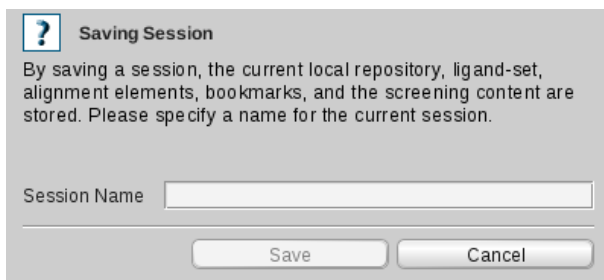


Figure 2.4. Save Current Session

Remove session

If you want to remove a specific session go to *File > Sessions* menu. A list of available sessions appears in the *Managing Sessions* dialog where you can delete a session of your choice.

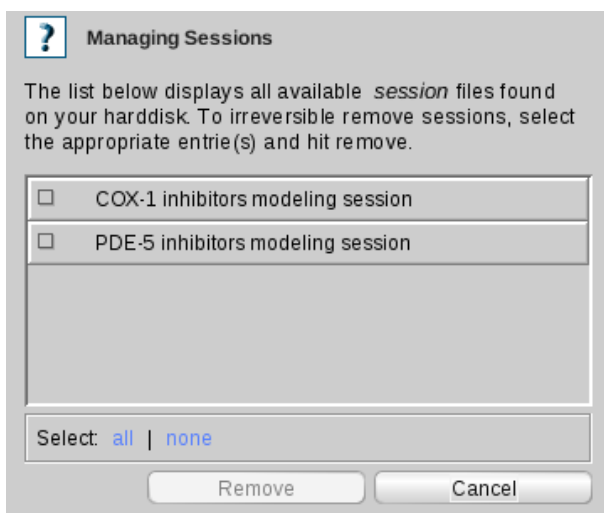


Figure 2.5. Managing sessions

Restore a session

You can load a previously exported session file through the menu *File > Sessions > Load Session from File*. Alternatively, choose a session from a list of available sessions provided in the *Sessions*

submenu. Note that the new session replaces the current one. Thus, all of your current data from the Local Repository, Ligand-Based Modeling Perspective, Alignment Perspective, Bookmark View and Screening Perspective will be getting lost.

2.5. Networking

Computational chemistry is a field where the execution of tasks can take a lot of time. Especially if huge molecule databases are used: e.g., to generate conformations or screen them against target pharmacophore(s), getting the results can take several hours, days or even months. Scientific and innovative solutions are needed to face this problem. Since hardware is getting cheaper and cheaper and processors exhibit more and more cores, execution time of tasks can be dramatically reduced.

Thus, LigandScout makes use of multiple cores to split up tasks into multiple small tasks that can be executed in parallel. But there is more to it than that. LigandScout comes with a whole framework to execute these small tasks not only on multiple cores on a single machine, but in a network on multiple computers using all resources available to speed up execution.

2.5.1. iworker

To use computers connected through the network, the so called “iworker” has to run on each machine in the network. The iworker is a stand-alone application that can be started either in the [command-line or as a graphical user interface application](#). Each iworker can execute multiple tasks in parallel to take full advantage of multi-core and/or multi-processor systems. While the iworker is running, it listens through a network port for incoming tasks. This port needs to be specified in the [iworker Settings](#) and [Preferences/Distributed Calculation Settings](#). Make sure that the system administrator has opened that port! To keep an overview about the iworker in the network, they are monitored by the [Network Monitor](#). Once the iworker receives a task it executes it and sends the result back to LigandScout. LigandScout collects all the results of the small tasks and displays the progress and the overall result.

You can stop an iworker whenever you want. Results will never get lost, even if the machine running the iworker has crashed unexpectedly. Tasks which were in execution, just when the iworker stopped or crashed, will simply be restarted on another iworker. Furthermore, iworkers can be started on the fly. LigandScout will make use of them as soon as they are started.

To use this opportunity of distributed calculations, you need to enable this mode in the [Preferences/Distributed Calculation Settings](#). iworkers that are used by LigandScout can be added or removed in the [list of iworkers](#) in the Preferences. If a listed iworker is not running or cannot be connected to, LigandScout will regularly retry to connect. In the meantime all available iworkers will be used. Optionally, LigandScout can find iworkers automatically, if they run on a LAN (Local Area Network) by using UDP-Multicast (This works only if broadcasting is enabled by the system administrator!).

To prevent idle time between finished tasks and new incoming tasks, which would cost performance, the iworker takes more tasks than it can execute in parallel and puts them into a so called “Work Queue” (queued tasks). Whenever a task execution has finished the iworker starts to execute the next task in the queue with no idle time in between. Preventing idle time as much as possible increases the execution performance.

How to start the iworker

The iworker can be started in two ways: The [stand-alone GUI](#) and through the [command line](#).

The stand-alone GUI

Press on the iworker application icon to start the iworker GUI. It consists of an information overview about the iworker, option settings to be adjusted to your needs and a button that starts/stops it.

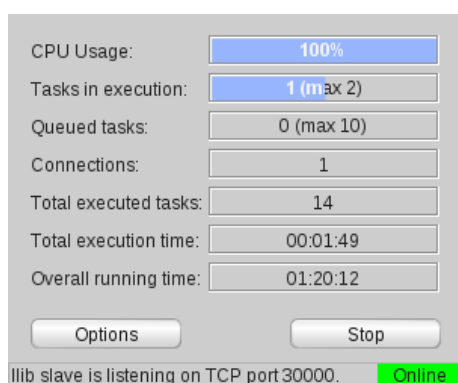


Figure 2.6. iworker

iworker Information

This information gives an overview of the status of the iworker. The *CPU Usage* shows the overall processor load of the machine running the iworker. The number of tasks that are currently processed are displayed in the *Tasks in Execution* field. The next field called *Connections* represents the number of nodes (LigandScout) that are connected to this particular iworker. The value of the *Queued Tasks* informs you about the number of tasks in the *Work Queue*. The *Total Executed Tasks* implies the number of tasks that are executed so far. Whereas, the *Total Execution Time* is the time that the iworker spends for executing the tasks. The *Overall Running Time* field shows the sum of idle time and the execution time (time between starting and stopping the iworker). Note that restarting the iworker does not reset the number of tasks, total execution time, and overall running time, but continues the counting.

iworker Options

Pressing the *Options* button, a dialog appears for changing the iworker settings.

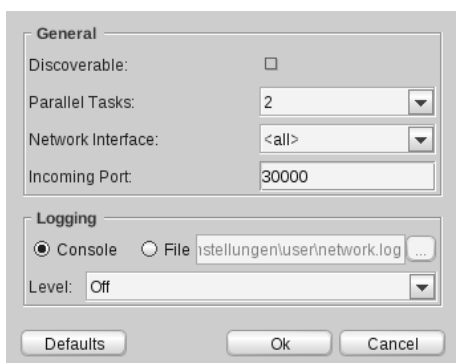


Figure 2.7. iworker Options

If the *Discoverable* setting is enabled, the iworker can be detected in the network. If disabled, the only way to find this iworker is to define it in the [iworker list](#). This list consists of addresses of the iworkers and are specified in the LigandScout [Preferences/Distributed Calculation Settings](#) chapter. To set the maximum number of tasks executed in parallel, just fill an appropriate value in the *Parallel Tasks* field. This number is limited to the available number of cores (e.g. maximal 2 on a dual-core system, maximal 4 on a quad-core system). In the *Network Interface* field you can select the network device to use. The *Incoming Port* represents the network port where the iworker listens for incoming tasks. This port has to be opened from your system administrator (TCP port). Note that if the options settings of the iworker are changed, during the iworker runs, the iworker has to be restarted to make these changes active.

The logging messages of the iworker can be shown in the console or redirected to a specified file. In the drop down box you can select the logging level which defines how much information of the log will be provided. Raising the level leads to a more detailed output. Choose between *off*, *low*, *medium*, *high* and *highest*.

Log Level	Description
off	no network log messages
low	network errors and warnings
medium	connection info messages are shown
high	high-level protocol messages
highest	debug (very verbose)

2.5.2. Network Monitor

The *Network Monitor* can be used to observe the iworkers in the network. The iworkers can be detected in two ways. You can specify the network addresses of the iworkers in the [appropriate list](#) of the Network Monitor Preferences. Secondly, iworkers are automatically found in the LAN when the [Auto-detectable Option](#) of the iworker is activated and provided that the broadcasting are enabled.

The Network Monitor consists of an [iworker Information Table](#), that provides a detailed overview about the iworkers and a [Log View](#) for error handling.

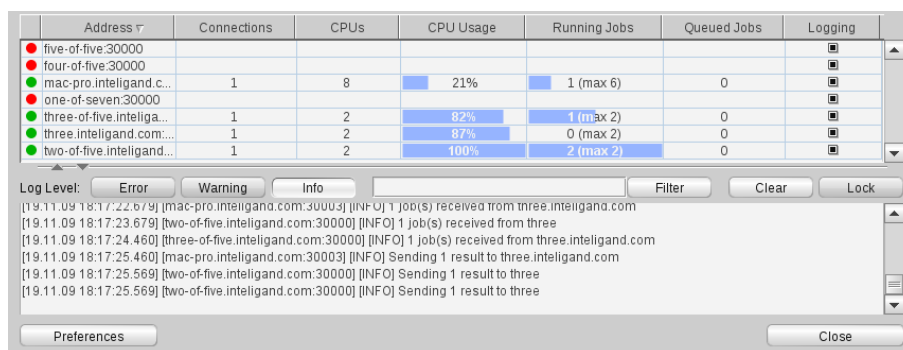


Figure 2.8. Network Monitor

iworker Information Table

The *iworker Information Table* shows information about the current state of all iworkers found in the network. Each row represents an iworker and displays following information about that iworker: Network Addresses of the iworkers (*Addresses*), number of nodes (LigandScout) that are connected to this particular iworker (*Connections*), number of CPU's (*CPU*), CPU usage of the machine running the iworker (*CPU Usage*), number of tasks that are currently executed (*Running Jobs*), number of queued tasks (*Queued Jobs*) and Enabling [Log](#) to view errors, warnings and other information about the iworker (*Log*). Note: Disabling the Log decreases the network-traffic.

Log View

The *Log View* displays errors, warnings or other useful information about the iworker. The Log shows only entries of iworkers selected in the iworker Information Table. If no iworker is selected, entries are shown for all iworkers. Any selection in the iworker Information Table can be reseted by clicking on an empty area in the iworker Information Table.

The detail level of the Log can be changed by the three Log Level buttons "Error", "Warning", and "Info". The first level "Error" shows only the errors (bold and red). The second level "Warning" illustrates the warnings (bold) and errors (bold and red). The last level "Info" shows all information of the iworker including warnings and errors.

Furthermore, a text filter can be set to show only those entries that contain the specified text. To apply such a filter, type the text into the filter area and press the *Filter* button. To disable the text filter press the *Cross* button that is shown when a text filter is successfully applied to the Log. You can clear the Log by pressing the *Clear* button. Note: Incoming Log entries are kept in temporary files. These files are deleted when the Network Monitor is closed and whenever the *Clear* button is pressed. It is a good idea to clear the Log if the temporary files require too much hard disc space. Never delete the temporary files manually! If the space in the Log View is exceeded the Network Monitor automatically scrolls to the last incoming entry. Automatic scrolling can be enabled or disabled by toggling the *Lock* button.

Network Monitor Preferences



Figure 2.9. Network Monitor Preferences

In the Network Monitor Preferences dialog you can customize the logging settings and manage the list of iworkers in the network.

You can choose if the logging of the message traffic will be shown in the console or redirected to a specified file. In the drop down box you can select the logging level which defines how much information of the log will be provided. Raising the level leads to a more detailed output. Choose between *off* (no network log messages), *low* (network errors and warnings), *medium* (connection info messages are shown), *high* (high-level protocol messages) and *highest* (very verbose for debug).

Add or remove iworkers by adding or removing their network addresses. A network address has the form `HOSTNAME:PORT` or `IP_ADDRESS:PORT` (e.g.: `localhost:4283`, `135.27.1.119:1894`). `HOSTNAME` or `IP_ADDRESS` is the host name or ip-address of the iworker, and `PORT` is the TCP-port the iworker is listening to. (Remember that the system administrator has to open that port on the machine the iworker is running on!).

Chapter 3. LigandScout Perspectives

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LigandScout offers several solutions to handle complex problems in drug design using pharmacophore models. These approaches are divided into four perspectives:

1. [Structure-Based Modeling Perspective](#)
2. [Ligand-Based Modeling Perspective](#)
3. [Alignment Perspective](#)
4. [Screening Perspective](#)

The Structure-Based Modeling Perspective is divided into two views: PDB View and Bookmark View. The PDB View allows for analyzing the binding site of ligand-receptor complexes. Active sites including a part of the macromolecule, the ligand(s) and the pharmacophore can be stored in the Bookmark View for later investigation. If no 3D data of proteins are available the Ligand-Based Modeling Perspective offers possibilities to generate pharmacophores from a set of active compounds. In the Alignment Perspective, insight on the structural relationship of ligands as well as the fit of compounds to pharmacophores can be gained by aligning pharmacophores and/or molecules (single conformation). The Screening Perspective allows for pharmacophore-based virtual screening of large molecule databases.

The advantage of the perspective concepts is that you can simply switch between the four perspectives and exchange data from one to another. This makes LigandScout flexible in its application while the graphical user interface remains structured clearly. All perspectives include dedicated elements that are backed by several “Basic User Interface Modules”, such as a Hierarchy View, a 2D View, and a 3D View which provide basic information about your data.

3.1. Basic User Interface Modules

LigandScout is composed of Basic User Interface Modules and widgets which helps you to display and navigate through your data. Some of them appear in all five perspectives, such as [2D View](#), [3D View](#), [Hierarchy View](#) and [Copyboard Widget](#). Each perspective has individual control panels - for example: the [PDB Panel](#) in the Structure-Based Modeling Perspective. Special modules like the [Library View](#) are only available in the [Structure-Based Modeling Perspective](#) and [Screening Perspective](#).

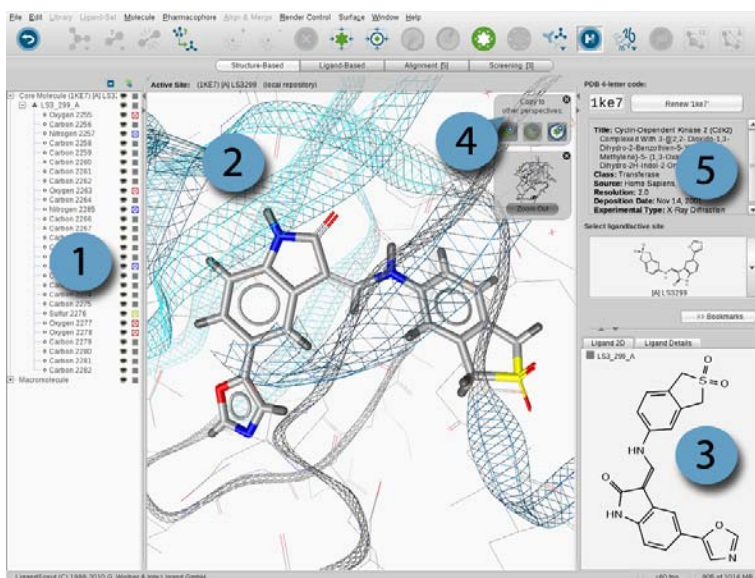


Figure 3.1. Basic User Interface Modules: Hierarchy View (1), 3D View (2), 2D View (3), Copyboard Widget (4) and control panel (5)

3.1.1. 3D View

The *3D View* represents the most important interaction of LigandScout. It is a powerful tool to verify and revise structural data, which is crucial for the automated generation of restrictive [pharmacophore models](#).

Working with the 3D View

Since PDB data does not provide information on atom hybridization states and bond types, LigandScout uses a sophisticated ligand interpretation algorithm for the assignment of bond types via molecular geometry analysis. Data quality of PDB ligands is known to be an issue in molecular modeling. Therefore, manual verification of the automatically deduced ligand structure is highly recommended. Moreover, it represents a vital verification step for the subsequent pharmacophore generation process.

Navigation Basics in 3D View

Position the mouse pointer in the *3D View* and press the LEFT mouse button to rotate the items shown in the 3D View. Depending on your hardware setup, scrolling your mouse wheel or pressing the MIDDLE/WHEEL mouse button and moving your mouse up/down allows you to zoom. The structure can be translated by pressing the RIGHT mouse button while moving. A comprehensive [list of mouse controls](#) is provided in the [Section A.4, "LigandScout Mouse Bindings"](#).

Selecting Elements of the 3D Viewer

Select a single element by a single LEFT mouse click on it. Keeping SHIFT pressed allows you to select consecutive elements, CTRL (Command on MAC) pressed lets you select individual items. The *Select* command in the *Edit* menu picks all elements of ligand, environment or pharmacophore. *Select All* is identical to hotkey CTRL+A (Command+A on MAC) and picks all items at once. Please refer to [Mouse Bindings](#) for more details on mouse interactions.

Displaying Essential Information on Selected Items

The [Toggle Tooltips](#) feature in the toolbar provides relevant characteristics of the currently selected item in a transparent frame adjacent to the selection:

Item	Characteristics
Atom	Geometry, position, compound name
Bond	Bond type, length, ring +/-
Pharmacophore Sphere	Type, position, tolerance
Pharmacophore Vector	Type, orig. position, targ. position

Changing Atom Types

Atom types are changed quickly by selecting an atom and pressing the character of the respective element in your keyboard (this works for C, N, O, S, P). Apart from using these hotkeys, the [Change Atom Type](#) button in the toolbar gives you full access to the periodic system, where you can pick the desired element.

Creating (New) Atom Bonds

To create a new bond between two atoms, keep SHIFT pressed and select two atoms. Then select the [Create New Bond](#) icon. If you want to change a bond type, select the bond and press 1 (SINGLE), 2 (DOUBLE) or 3 (TRIPLE) on your keyboard to change the current bond into the respective bond type. Additionally to these shortcuts, the [Change Bond Type](#) button in the tool bar provides this editing function.

Creating New Atoms

Create a new atom by selecting a free hydrogen atom of a ligand. Change the selected hydrogen atom to the atom of your choice. This procedure automatically adds the new atom to the ligand and roughly optimizes the structure in the neighborhood of the added atom to avoid sterical clashes. You can use the hotkeys CTRL+ALT+O (Command +ALT+O on MAC) to minimize the ligand until a local minimum is reached. For more information on this please see [Section 9.3.4, "Force Field: Minimization"](#).

Deleting Atoms, Bonds, and Pharmacophore Features

Select an atom, bond, or pharmacophore feature you want to delete and press the **DEL** button on your keyboard. The [Delete](#) button in the tool bar also provides this functionality. Multiple selection is enabled for all elements; e.g. you can select various atoms, bonds, and pharmacophore features for simultaneous deletion. See [Section A.4, "LigandScout Mouse Bindings"](#) for additional information on multiple selection.

Adding Distance/Angle Monitor

To measure the distance between two atoms, just keep SHIFT pressed and select two atoms. Select the *Add Distance Monitor* in the *Render Control* menu or the [Add Distance Monitor](#) icon in the tool bar. To measure the angle between three atoms, keep SHIFT pressed and select three atoms. Select the *Add Angle Monitor* in the same menu as above or the [Add Angle Monitor](#) icon. To delete the distance or angle measurement, click on the shown value and press the [Delete](#) icon.

Rotate around a Bond

To rotate a part of a molecule around a specific bond, select its two neighbored bonds on either sides and press on the Angle Monitor [Add Angle Monitor](#) icon. Select the angle value and keep the LEFT mouse pressed to rotate the bond. To rotate a bond on the distant side of the molecule, simply rotate the camera to bring the bond to the front.

Editing Charges

Correct charges are crucial for the generation of valid pharmacophore models. To adjust the charge of atoms, make a selection and use the [Increase Charge](#) / [Decrease Charge](#) buttons or in the menu bar *Molecule > Increase/Decrease Charge of Selected Elements* command. Please note, that *Increase/Decrease Charge* functionality is only available on high-resolution displays.

Defining Elements as Core or Environment

In special cases you may want to shift elements from core to environment and vice versa. Just select the atoms and/or bonds you want to move and use the *Move Selection to Macromolecule/Ligand Side*

command located in the *Molecule* menu. The respective buttons [Move to Environment](#) and [Move to Core](#) provide instant access to this LigandScout feature.

Returning to the Basic Structural Data

The [Re-Interpret Core Molecule](#) button lets you recalculate bond valences, aromaticity, hydrogen positions, and stereo chemistry from scratch. This feature is useful if you lose track of your modifications. However, if you only want to undo your last modification(s) you can use the *Undo* feature via the [Undo](#) button or in the menu *Edit > Undo*.

Advanced Render Control Settings

LigandScout provides useful render control options such as e.g. *Wide Angle View* and *Show Tool Tips*. Please refer to [the section called "Projection"](#) and [the section called "Behavior"](#) for more information.

3.1.2. 2D View

The *2D View* displays the structural formula of ligands and its generated pharmacophore.

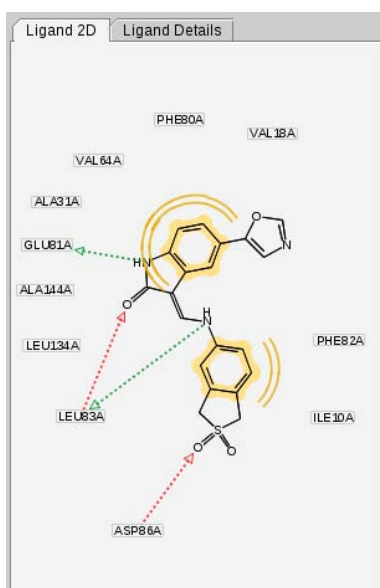


Figure 3.2. 2D View

Like its 3D counterpart, the 2D View is used for fast atom and bond type corrections. Furthermore, LigandScout now allows you also to edit the pharmacophore features directly in the *2D View* in the same way as described for the [3D View](#). Select one or several pharmacophoric constraints by single click on the respective residue identifier(s) located nearby the pharmacophore feature. There are no major differences in handling between both tools. Selections and manipulations are identical to that described for the *3D View*. Both the 2D depiction of the ligand and pharmacophore can be [exported in several file and graphics formats](#).

3.1.3. 3D Widgets

Copyboard Widget

The Copyboard Widget gives the opportunity to transfer your chemical data between different perspectives of LigandScout (Structure-Based Modeling, Ligand-Based Modeling, Alignment and Screening).



Figure 3.3. Copyboard Widget

You can toggle this widget on/off in the menu *Window > 3D Widgets > Copyboard Widget*. Molecules are added from one perspective to another using the [Copy current Ligand\(s\) to](#) icon. Pharmacophores are added with the [Copy current Pharmacophore to](#) icon, respectively. Exclusively active sites (binding pockets) can be transferred only from the Structure-Based Modeling Perspective to the Bookmark View by clicking on the [Copy current binding site to](#) icon.

Please be reminded that you can add items to the Bookmarks Panel only from the PDB View. In other words, you have to switch to PDB View of the Structure-Based Modeling Perspective to add the current molecule or pharmacophore to the Bookmarks Panel. The items can be placed individually within the 3D View.

Some examples: If an active site is currently shown, this allows you to add the ligand molecule to the Alignment Panel by clicking on the icon [Copy current Ligand\(s\) to](#) Alignment Perspective. If the pharmacophore was calculated you can add it to the Screening Perspective by clicking on the icon [Copy current Pharmacophore to](#) Screening Perspective. The transferred items can be placed individually within the 3D View.

The following table gives an overview of the items that can be exchanged between the perspectives.

From\To	PV	LBP	AP	SP	BV
PV	-	L, P	L, P	P	A
LBP	L, P	-	L, P	P	-
AP	L, P	L, P	-	P	-
SP	L, P	L, P	L, P	-	-
BV	L, P	L, P	L, P	P	-

Table 3.1. Exchanging data between perspectives: ligand/s (L), pharmacophore/s (P), active site (A), Structure-Based Modeling Perspective: PDB View (PV) and Bookmark View (BV); Ligand-Based Modeling Perspective (LBP), Alignment Perspective (AP) and Screening Perspective (SP)

Alternatives Switcher

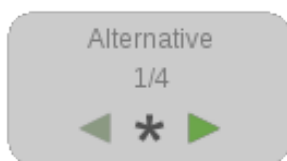


Figure 3.4. Alternatives Switcher

In case of navigating within your molecule library, select more than one compound in the [Library View](#) and the Alternatives Switcher automatically appears. The widget allows you to visualize the previous ([Show Previous Compound](#)), next ([Show Next Compound](#)) and all ([Show All Compounds](#)) visible compound(s). The Alternatives Switcher can be de/activated in the menu *Window > 3D Widget*.

Conformation Switcher

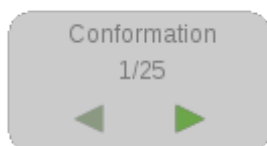


Figure 3.5. Conformation Switcher

The Conformation Switcher navigates you through the conformations of your selected compound. The widget automatically appears if conformations are available and can be toggled in the menu *Window > 3D Widgets*.

Tautomer Switcher

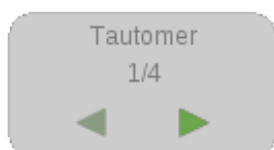


Figure 3.6. Tautomer Switcher

You can switch through available tautomers of a compound by using the Tautomer Switcher. The widget can be toggled in the menu *Window > 3D Widgets*.

3.1.4. Hierarchy View

The *Hierarchy View* shows a tree diagram of the current macromolecule-ligand complex as you are used from various file commanders. A click on the '+' box on the left of the branch title opens a sublevel.

The [Collapse All](#) button lets you return to the starting view of this display. To toggle the visibility of an element click on the [Toggle Visibility](#) icon. The [Change Color](#) icon allows you to change the element color.

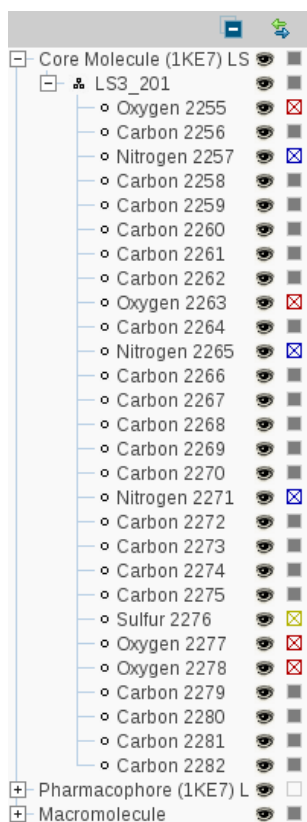


Figure 3.7. Hierarchy View

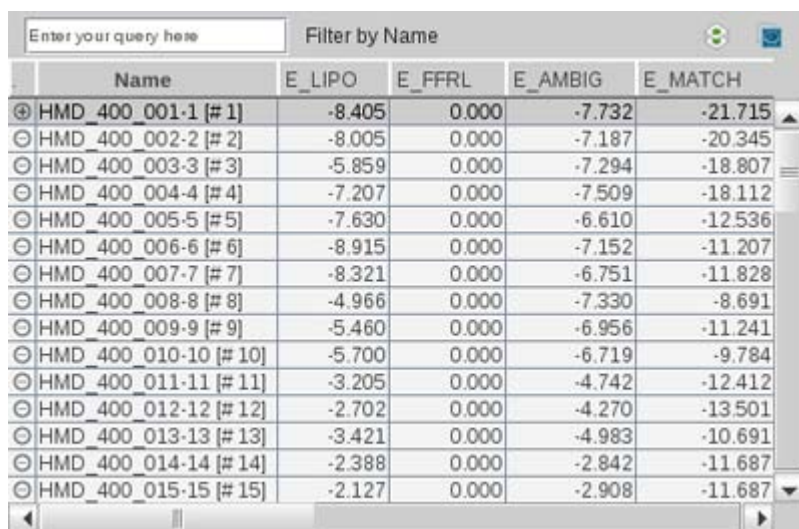
Selecting Items with the Hierarchy View

A single LEFT mouse click on a branch title selects all elements of all subordinate levels. Keeping SHIFT pressed allows you to select consecutive elements, CTRL pressed lets you select and deselect individual items.

The [Link with Selection](#) icon connects the selection in the 3D View to the Hierarchy View. As you select items in the 3D View with this option enabled, Hierarchy View opens all necessary sublevels to focus and highlight the currently selected items.

3.1.5. Library View

The *Library View* allows you to efficiently investigate collections of small organic molecules. It is especially useful for the analysis of pharmacophore screening and docking results.



Name	E_LIPO	E_FFRL	E_AMBIG	E_MATCH
⊕ HMD_400_001-1 [#1]	-8.405	0.000	-7.732	-21.715
⊖ HMD_400_002-2 [#2]	-8.005	0.000	-7.187	-20.345
⊖ HMD_400_003-3 [#3]	-5.859	0.000	-7.294	-18.807
⊖ HMD_400_004-4 [#4]	-7.207	0.000	-7.509	-18.112
⊖ HMD_400_005-5 [#5]	-7.630	0.000	-6.610	-12.536
⊖ HMD_400_006-6 [#6]	-8.915	0.000	-7.152	-11.207
⊖ HMD_400_007-7 [#7]	-8.321	0.000	-6.751	-11.828
⊖ HMD_400_008-8 [#8]	-4.966	0.000	-7.330	-8.691
⊖ HMD_400_009-9 [#9]	-5.460	0.000	-6.956	-11.241
⊖ HMD_400_010-10 [#10]	-5.700	0.000	-6.719	-9.784
⊖ HMD_400_011-11 [#11]	-3.205	0.000	-4.742	-12.412
⊖ HMD_400_012-12 [#12]	-2.702	0.000	-4.270	-13.501
⊖ HMD_400_013-13 [#13]	-3.421	0.000	-4.983	-10.691
⊖ HMD_400_014-14 [#14]	-2.388	0.000	-2.842	-11.687
⊖ HMD_400_015-15 [#15]	-2.127	0.000	-2.908	-11.687

Figure 3.8. Library View

Importing Libraries

Compound collections can be imported to the *Library View* using the *Open* or *Insert* command in the *File* menu. The LigandScout I/O framework currently supports MDL SD, SMI, MOL2 and LDB file formats for loading molecule libraries.

The *Open* command is used to display the molecule exclusively in the *Library View*. Select a file containing the molecules you want to analyze with LigandScout and click *Open File*. LigandScout now imports all ligands, activates the *Library View* and shows all properties contained in the loaded library. Opening libraries with no 3D coordinates such as SMI files (or also SD(F) possible) displays the compounds only in 2D.

The *Insert* command (CTRL+I) can be used in two ways: You can open a file containing an particular ligand and/or a pharmacophore and then insert a library. The second possibility is to load a PDB code and zoom into the binding site to insert the library. The loaded data have to be in the correct coordinate frame to yield a meaningful result. For instance, if the molecule to be injected was docked into the active site before, this restriction will be fulfilled. If the coordinate frame is not correct, the inserted ligand will be placed into the coordinate frame of the active site whether it makes sense or not.

Navigate in the Library View

In order to view compounds of your collection, make a single click on a compound row of the library to visualize it. To extend your selection, press CTRL and choose additional compounds. The scroll bar on the right side lets you navigate through your compound collection.

If you select more than one molecule in your library, you can navigate within your selection and show the molecules one by one. Thus, the [Alternatives Switcher](#) is automatically activated and you can switch to your previous, next or all molecule(s). The widget can be toggled in the menu *Window > 3D Widgets*.

In concordance to the handling within the [Hierarchy View](#) you can also select several ligands for visualization. The visibility of the library compounds can be toggled with the [Toggle Visibility](#) icon in the

Hierarchy View. According to a library insertion into a binding pocket, the *original* ligand (core molecule) remains visible (if existing) in the binding site unless it is erased or defined [hidden](#) in the [Hierarchy View](#). This is especially useful if you e.g. want to compare your docking results to a binding pose of a known active compound.

If you add a molecule from another view to the PDB View, a pop up dialog asks if you want to *Append to Library*, *Replace Core Molecule* or *Inject Only*. The latter one adds the molecule only to the Hierarchy View.

You can arrange the property columns of the Library View individually by moving the column identifiers. Click on a column identifier (e.g. *Name*) to sort the table by this property. Another click on the same column identifier flips the sort order. For instance you could sort your docking/pharmacophore screening results by decreasing score. In this way you can use LigandScout to investigate your virtual screening results ranked by relevance.

To toggle on/off specific column identifier, click on a column identifier using the RIGHT mouse button. A list of available identifiers appears where you can enable or disable columns by de/selecting their appropriate check box.

On the upper left corner of the Library View LigandScout allows to apply filter criteria to the compound table: Press CTRL and click on a column identifier to select the parameter you want to use for filtering (default: Name). Subsequently, click on the *Add Filter* label and a text box appears. When you enter a filter criterion, the table will be updated instantly, considering the currently applied criterion. To undo the filtering erase the filter criterion in the text box.

Hide all compounds of the Library View with the [Hide All Compounds](#) icon located on the upper right corner of the *Library View*. Next to this icon you find the [Align Molecules on Injection into the Binding Site](#) icon, which allows you to directly align the selected molecule(s) to the active site by overlapping the feature tolerances. Enabling *Align selection to Pharmacophore/Core Molecule* aligns and scores the selection to the context.

3.2. Structure-Based Modeling Perspective

The Structure-Based Modeling Perspective offers a user interface ready to explore PDB complexes and to derive pharmacophores from the binding sites. This perspective is divided into two views: the PDB View and the [Bookmark View](#).

3.2.1. PDB View

Besides [loading data from file](#), a very convenient way to obtain ligand-macromolecule complexes is to directly [retrieving PDB complexes online](#). This task can be performed by using the dedicated control panel, the *PDB Panel*.

Another important use covered by the Structure-Based Modeling Perspective is investigating small-molecule libraries. This can be done either with or without a particular binding site as context by using the [Library View](#).

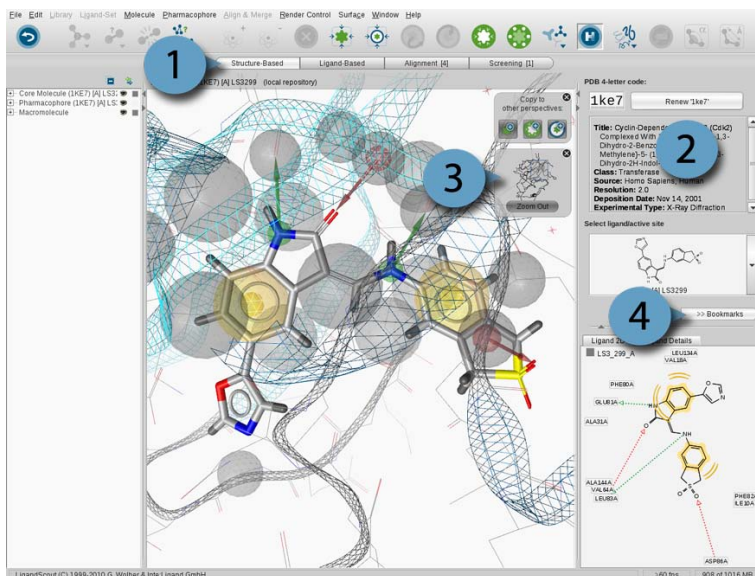


Figure 3.9. The PDB View of the Structure-Based Modeling Perspective (1): PDB Panel (2), Zoom Out Widget (3) and Bookmark View button (4)

PDB Panel

The *PDB Panel* displays important information on the current macromolecule-ligand complex, provides instant PDB access and is composed of three components:

- PDB 4-letter Code Instant Access Box
- Essential characteristics of the PDB file
- Ligand Selector

PDB 4-letter code:

1ke7

Title: Cyclin-Dependent Kinase 2 (Cdk2)
Complexed With 3-[[[2,2-Dioxido-1,3-Dihydro-2-Benzo[thien-5-Yl]Amino]Methylene]-5-(1,3-Oxazol-5-Yl)-1,3-Dihydro-2H-Indol-2-One

Class: Transferase
Source: Homo Sapiens, Human
Resolution: 2.0
Deposition Date: Nov 14, 2001
Experimental Type: X-Ray Diffraction

Select ligand/active site

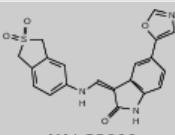

[A] LS3299

Figure 3.10. PDB panel

LigandScout uses a "two-level" file storing strategy, the [Local and Shared Repository](#). If a PDB code is entered in the *PDB 4-letter code field* of the PDB Panel, LigandScout tries to find the associated files in the Local Repository, the first stage. If unsuccessful, LigandScout will check the Shared Repository which represents the second level.

Title, class, source, resolution, deposition date, and the experimental type of the current PDB complex are provided in a separate text box.

The *Ligand Active Site Selector* allows instant access to the active sites of the current complex. Simply click the drop-down menu and select a ligand to zoom into the respective active site. If you are already in an *Active Site View* the view will be switched without an animation.

3D View Depiction Modes and the Zoom Out Widget

LigandScout's *3D View* provides two distinctive macromolecule-ligand complex depiction modes in the Structure-Based Modeling Perspective. Startup sets the display to *Macromolecule View*. LigandScout memorizes views and positions. Thus, if you change from [Active Site View](#) to [Macromolecule View](#) using the *Zoom Out Widget* you will get your previous view setup of the current display. The visibility of the *Zoom Out Widget* can be toggled in the menu *Window > 3D Widgets*.

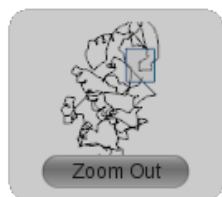


Figure 3.11. Zoom Out Widget

The *Macromolecule View* illustrates the global structure and current orientation of the macromolecule. You can switch to the active site by a LEFT mouse click on the active site (blue box). To change back

to the *Macromolecule View* just click on *Zoom out* button of the *Zoom Widget*. The *Zoom Widget* can be placed individually within the *3D View*.

Macromolecule View

The *Macromolecule View* provides structural information on the macromolecule and the ligand's position within it. This view gives you a clearly arranged overview on the current PDB complex. Alpha-helices are shown in dark blue, beta-sheets in light blue, and double helices in brownish-orange. Consecutive amino acids which could not be categorized are shown in gray. If some amino acids or nucleic acids are missing, a gray dashed line will be shown.



Figure 3.12. Macromolecule View

Use your mouse to navigate within the *3D View*. A single click on [Center Ligand and Environment](#) in the toolbar lets you return instantly to the *Macromolecule View* starting position.

In *Macromolecule View*, four different rendering modes of both macromolecule and ligand are available. Use the *Ligand Default Style* submenu of the *Render Control* menu or the [Change Render Style of Selected Atoms](#) icon to change between *Line*, *Ball and Stick*, *Stick* and *Spacefilling/CPK* ligand rendering mode. Similarly the rendering mode of the macromolecule is changed with the *Protein Backbone Default Style* submenu of the *Render Control* menu or the [Toggle Hydrogen](#) button. LigandScout supports *Lines*, *Splines*, *Snake* and *Ribbon* styles.

Active Site View

The *Active Site View* displays structural information of the ligand and its essential surrounding macromolecular environment in detail. LigandScout displays only amino acids or nucleic acids surrounding the

ligand while suppressing moieties irrelevant for macromolecule-ligand interaction. The respective cutoff value is defined in the Preferences/Structure-Based Modeling Settings as [Interaction Cutoff Threshold](#).

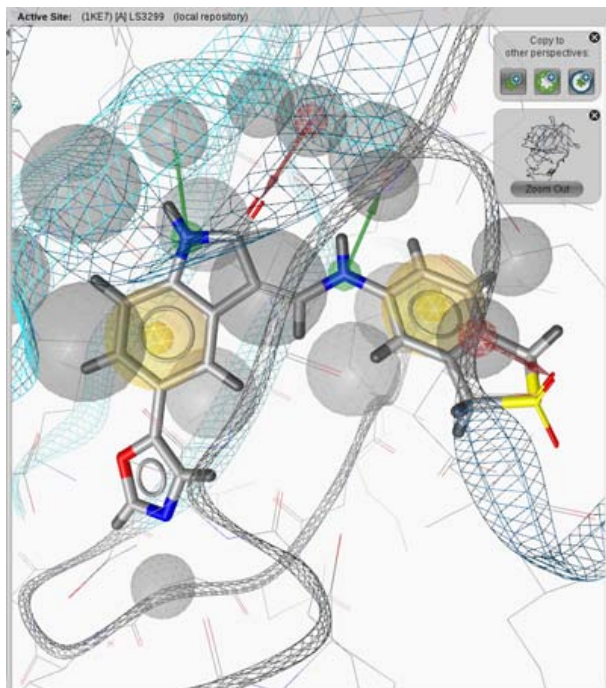


Figure 3.13. Active Site View

You can easily switch from *Macromolecule View* to *Active Site View* by selecting a ligand in the *Ligand Active Site Selector (PDB Panel)* or by selecting a yellow cube surrounding the ligand in the [3D View](#). Use the [Center Ligand Molecule](#) icon in the toolbar to recenter the ligand. [Center Ligand and Environment](#) button lets you recenter the current active site. A click on the *Zoom out* button of the *Zoom Out Widget* in the 3D View lets you return to the starting position in the *Macromolecule View*.

Rendering Options of the Active Site View

The *Active Site View* provides additional rendering options as available in *Macromolecule View*. The additional render options are found in the [Render Control menu](#).

Ligand Rendering Settings: Ligand Default Style Submenu

Toggle on/off hydrogen depiction with the *Show/Don't Show Hydrogens* radio buttons or the [Toggle Hydrogen](#) icon. *Show Hydrogens Stubs* reduces the size of hydrogens to stubs. *Show Templates* activates the depiction of default geometric templates depending on the hybridization-state of the currently selected atom. *Show Interactions* displays all possible interactions of the currently selected atom. *Show Ionic Interactions* displays ionic interactions between metals and non-metals. The *Pickable* check box sets the ligand selectable/unselectable.

Environment Rendering Settings: Environment Default Style Submenu

The *Environment* rendering submenu corresponds in large part to the respective ligand menu and lets you select different rendering modes and define hydrogen handling. The *Show Templates* option activates the depiction of default geometric templates depending on the hybridization-state of the current-

ly selected atom. The *Pickable* check box sets all of the atoms in the environment as selectable/unselectable.

Selection Rendering Settings: Selection Submenu

The *Selection* submenu lets you customize the rendering of the current selection. LigandScout provides four different modes: *Line*, *Ball and Stick*, *Stick* and *Spacefilling/CPK*.

Pharmacophore Rendering Settings: Pharmacophore Submenu

The *Pharmacophore* submenu enables you to switch on/off pharmacophore and [excluded volume](#) depiction or use the [Toggle Exclusion Volume Spheres Visibility](#) icon. The *Pickable* check box sets the pharmacophore selectable/unselectable.

GRID Maps Visualization

The Structure-Based Modeling Perspective of LigandScout allows you to visualize GRID maps directly in the protein binding site. To import GRID maps into the binding site, select in the menu *File > Open*. LigandScout will ask you for the kind of GRID probe to be visualized.

The GRID maps are especially useful for the investigation of LigandScout pharmacophores. These maps provide information on the importance and intensity of certain protein-ligand interactions and may help you to discriminate between crucial pharmacophore features and constraints that are less important. Pharmacophoric features located in areas that indicate strong molecular interactions by GRID analysis (using compatible probes of course) are likely to be highly relevant for the binding of the ligand to the protein.

Since GRID maps do contain absolute coordinates, you need to load the appropriate binding site in order to have the GRID map correctly aligned with the pocket. At best, load the particular binding the GRID map has been calculated for.

3.2.2. Bookmark View

The Bookmark View stores active sites of macromolecules. This has the advantage that you can analyze the binding sites of interest one after the other without the need of loading them through the PDB Panel. The active sites are managed in the Bookmarks Panel.

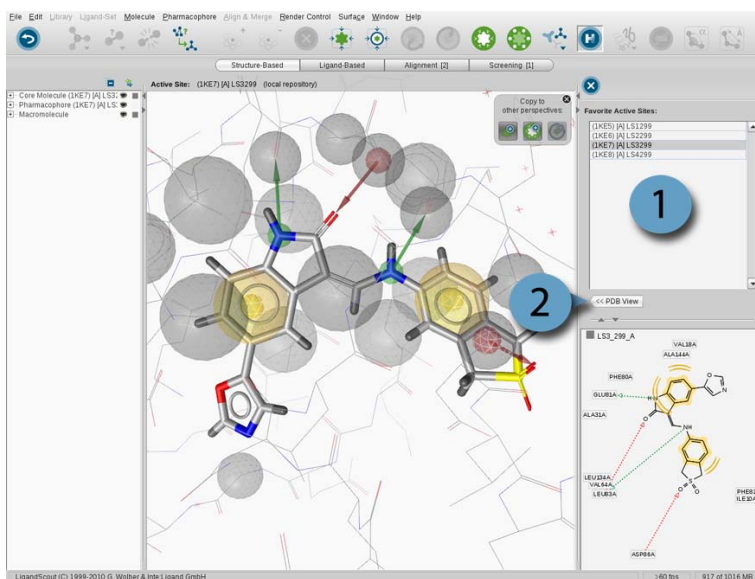


Figure 3.14. The Bookmark View of the Structure-Based Modeling Perspective: Bookmarks Panel (1) and PDB View button (2)

Bookmarks Panel

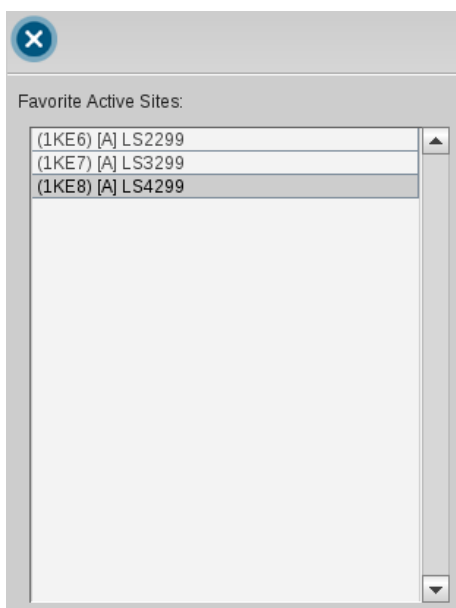


Figure 3.15. Bookmarks

The *Bookmarks Panel* allows you to access your bookmarked active sites which are represented by the [Bookmark](#) icon. Bookmarks of the current [Active Site View](#) are stored using the [Copy current binding site to](#) icon located on *Copyboard Widget* of the the Structure-Based Modeling Perspective. For instance, load 1ke6 into the Structure-Based Modeling Perspective and zoom in. Add the active site to the Bookmark View by the [Copy current binding site to](#). Repeat this procedure for 1ke7 and 1ke8. To access a bookmarked active site simply click on its name in the Bookmark List and the appropriate active site is shown in 3D View. You can erase a certain bookmark entry by selecting it and press the [Delete Bookmark](#) button.

3.3. Ligand-Based Modeling Perspective

Pharmacophore model creation strongly depends on the PDB complex when you choose the Structure-Based Modeling method. If no structural data of the protein-ligand complex is available or the complex lacks quality, then the Ligand-Based Modeling method is used. LigandScout's Ligand-Based Modeling Perspective allows you to generate pharmacophores from a set of ligands that bind to one target protein and provide similar biological activity (so called active ligands). LigandScout provides techniques which help to prepare an active set by clustering, include molecule flexibility by conformational analysis and an efficient alignment algorithm to derive pharmacophore models from a ligand-set.

The Ligand-Based Modeling Perspective includes [Basic User Interface Modules](#), a Ligand-Set Table and Results Table.

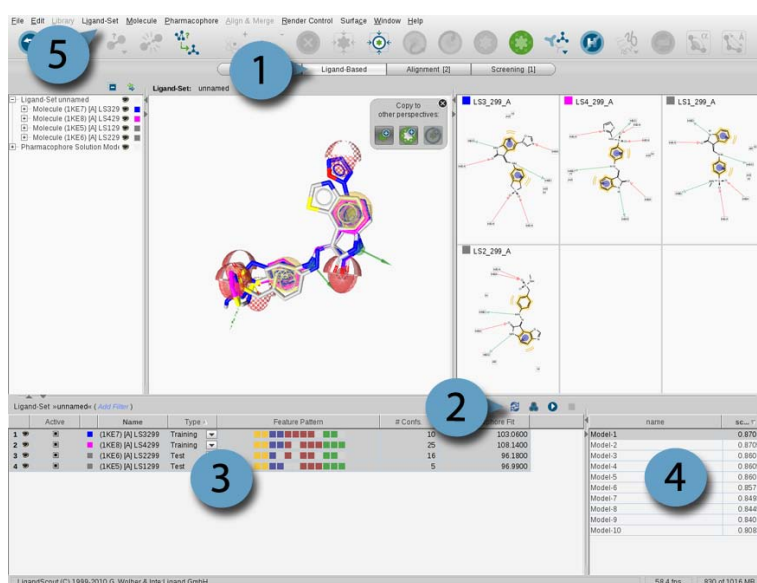


Figure 3.16. Ligand-Based Modeling Perspective (1), Tool bar (2), Ligand-Set Table (3), Results Table (4) and Ligand-Set menu (5)

3.3.1. Importing Ligands

Ligand-based pharmacophore modeling requires a set of two or more input ligands to generate characteristic pharmacophores. There are three different types of input ligands: Training-Set, Test-Set and Ignored Ligands. The Training-Set molecules are used for the actual pharmacophore creation and the Test-Set ligands are used to verify the resulting pharmacophores. Ligands that should not be included in pharmacophore generation and testing can be marked as Ignored Ligands.

The input ligands may be provided in three different ways: You can [import](#) e.g. a ligand set file (*.lsd) or add molecules to the existing Ligand-Set by selecting the *Add Molecules* submenu in the *Ligand-Set* menu and choose the type of set to be added. Another alternative is to add your molecules by means of the [Copyboard Widget](#) to the Ligand-Based Modeling Perspective.

3.3.2. Navigation and Working with the Tool Bar

Ligand-Set Table

The navigation in the Ligand-Based Modeling Perspective is quite similar to the [Library View](#), but with a different focus. The Ligand-Set Table (after importing the ligands) shows the list of molecules which serve as input for the pharmacophore generation process. The user can change ligand properties (e.g. type, activity, etc.), [filter \[33\]](#) the table content, and select ligands to make them visible in the 3D (only if 3D coordinates are present), 2D, and Hierarchy View. Like in the [Library View](#), navigating through available conformations is possible by using the [Alternatives Switcher](#).

The ligands are colored differently to keep the overview. The Training-Set ligands are colored randomly and ligands from the Test-Set and Ignored Ligands are marked gray. The color of the ligands can be changed in the [Hierarchy View](#). You can customize the type of a selected ligand by choosing the appropriate type in the Ligand-Set Table or flag the ligand in the *Ligand-Set* menu. It is also possible to change the activity of a ligand in this way.

In the Ligand-Set Table toolbar, buttons are provided for the main tasks in ligand-based pharmacophore modeling. From left to right: ligand conformations can be created by OMEGA ([Generate Conformations for Ligand-Set](#) icon), the available ligands can be clustered according to a multi-conformational alignment score ([Clustering](#) icon), and the automatic ligand-based pharmacophore creation ([Run Ligand-Based Pharmacophore Creation](#) icon) can be started. If conformations are already available, the pharmacophore generator uses them directly for the pharmacophore creation, otherwise they are generated on the fly. Conformer generation, clustering and pharmacophore creation can be customized as described in [Section 9.2, "Ligand-Based Modeling Settings"](#) and [Section 9.5, "Alignment Settings"](#).

When a ligand-based pharmacophore creation run is successful, the Ligand-Set Table is automatically updated and provides additional information such as feature patterns, number of conformations, and the Pharmacophore Fit of the ligands matched with the selected result pharmacophore. The first digit of the Pharmacophore Fit score represents the number of matched features and the second one, the RMSD value. The Feature Pattern of a ligand illustrates the color-encoded features that were matched by the ligand. Thus you can see at first glance the distribution of the features and which of the resulting pharmacophores are matched best by the Ligand-Set (frequency of matched features).

Results Table

The Results Table is positioned at the right-bottom corner of the Ligand-Based Modeling Perspective. After the ligand-based pharmacophore generation process has finished the results are listed in this table including the name and score. By default, the entries are sorted by score value in descending order, but the user can change both the sorting order and sorting criteria by clicking the appropriate column identifier.

Each entry of the Results Table represents a valid pharmacophore model (i.e. it consists of at least three features) and stores the state of all Training-Set molecules (i.e. alignment pose and active conformation). Selecting a resulting pharmacophore model updates the 3D View and 2D View to show the particular

pharmacophore and aligned Training-Set molecules. Furthermore, score values and feature patterns in the Ligand-Set Table are updated for the currently selected pharmacophore.

The range of the score values shown in the Results Table depends on the scoring function used (see [Ligand-Based Modeling Settings](#)). If you select the Pharmacophore Fit scoring function then the score value describes the number of matched features and RMSD. All other scoring functions produce normalized values that range from zero to one, where one is the optimum.

The generated ligand-based pharmacophores may be transferred into other perspectives, where you can [export](#) them in different file formats. Optionally, you can generate ligand-based pharmacophores by using the [command line](#) as well. For further information, please see [Chapter 7, Ligand-Based Pharmacophore Design](#).

3.3.3. Biasing Pharmacophore Generation

Ligand-based pharmacophore creation can be influenced in two ways. The first one sets a user-defined pharmacophore model to the starting point of the alignment procedure. The second directs the generation procedure by customized feature definitions.

User Defined Pharmacophore

During the ligand-based pharmacophore modeling process, ligands are ranked by their number of conformations. Then, starting from the ligand with the lowest number of conformations, their pharmacophore models are aligned with the ones of the second ranked ligand. Ligand-based pharmacophores will be composed from these alignments and aligned with the pharmacophores of the next ranked ligand and so on. If you set a user-defined pharmacophore in advance, this pharmacophore is kept as the first pharmacophore to be aligned to the pharmacophores of the following ligand. The advantage of this functionality is that the features of the user-defined pharmacophore are more likely to survive this procedure.

You can use this functionality by selecting in the menu *Ligand-Set > Set Pharmacophore Bias* and load a pharmacophore of your choice. Alternatively, you can add a pharmacophore from another perspective to the Ligand-Based Modeling Perspective using the [Copyboard Widget](#).

User Defined Feature Definitions

Although, the first functionality stamps the ligand-based pharmacophore modeling by a user-defined pharmacophore model, LigandScout allows you to provide additional customized feature definitions in addition to LigandScout's standard feature definitions (built-in types). These chemical features will be used temporarily for aligning the molecule, but will not be included in the resulting 3D pharmacophore. You can access the custom feature functionality directly in the *Ligand-Set* menu or via the settings dialog after clicking the [Run Ligand-Based Pharmacophore Creation](#) button in the Ligand-Based Modeling Perspective.



Figure 3.17. Customize feature definition

A custom feature is defined by several SMARTS patterns that specify the substructures where the feature shall (or shall not) be placed. For the definition of a new custom feature click on the *New* button under the available Features panel and specify an ID (abbreviation) and name for the feature. To enable/disable the feature in the pharmacophore generation process click the *Active* check box in the feature table.

To define or include SMARTS patterns for a feature click on the *New* button under the Include Patterns List. Declare valid SMARTS patterns and specify tolerance spheres for the feature. Smarts patterns that specify substructures where the particular feature shall not be placed (exclude patterns) are defined in the same way except that you do not need to specify a tolerance.

If you want to make changes to your custom feature definitions, you can edit them by clicking on the *Edit* button. To delete custom features as a whole or to delete only some include/exclude patterns of a feature, select the desired features or patterns and click on the appropriate *Delete* button. To apply your changes click the *OK* button on the bottom-right corner of the *Edit Custom Features* dialog. To reset any of your changes click on the *Reset Changes* button.

Use the custom feature functionality when you want to put emphasis on certain common substructures of the ligands in the alignment steps performed during the pharmacophore generation process. Note that although the custom features will be considered in the pharmacophore creation process (if enabled) they are not visible in the resulting 3D pharmacophores.

3.4. Alignment Perspective

The purpose of the Alignment Perspective is to analyze common properties (features or reference points) of pharmacophore models and/or small molecules by aligning them and generating shared or merged feature pharmacophores. Thus, the Alignment Panel and Alignment List help to manage your elements in the Alignment Perspective.

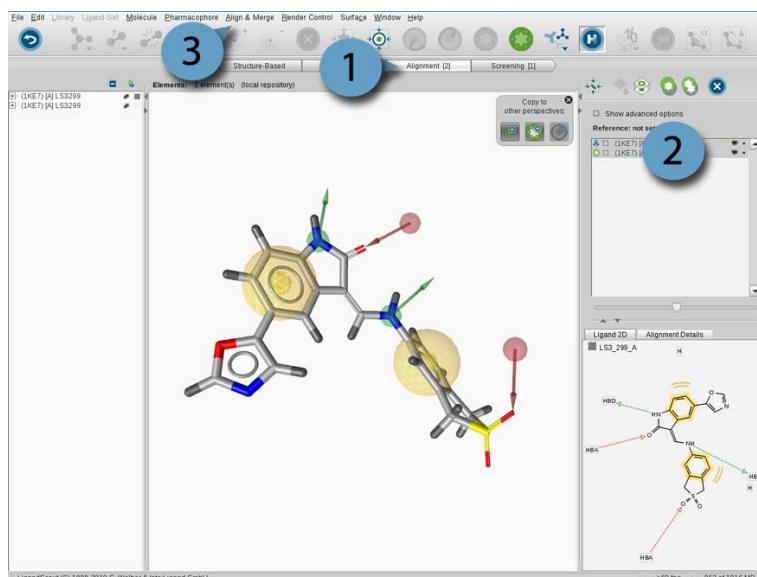


Figure 3.18. Alignment Perspective (1), Alignment List (2) and Align & Merge menu (3)

3.4.1. Alignment Panel

The *Alignment Panel* allows you to align pharmacophores and/or molecules as well as generate shared/merged feature pharmacophores.

Molecules and pharmacophores from other perspectives are added to the *Alignment Panel* using the *Copyboard Widget* or through the menu *File > Alignment > Import*.

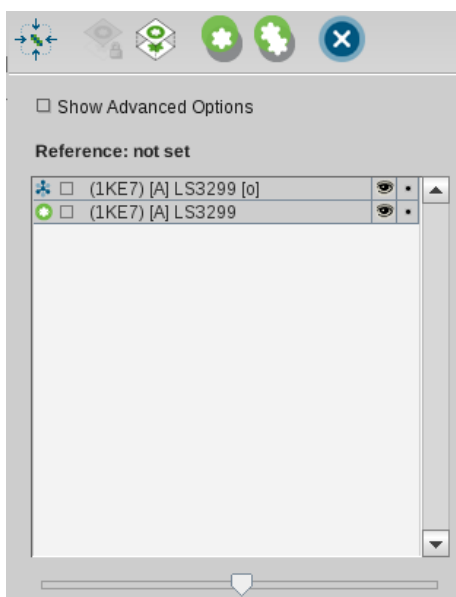


Figure 3.19. Alignment Panel

The *Alignment Panel* consists of an icon toolbar, the *Show Advanced Options Check Box*, the Alignment List containing molecules and pharmacophores, a slider, and an RMS label.

Icon Toolbar

Use [Center All Visible Structures](#) to move the center of all currently visible structures to one point in 3D space. To set a reference element, perform a list selection on a single element in the Alignment List and click [Set Reference](#). Clicking this icon again will reset a previously set reference element. To perform an alignment, select two or more elements from the Alignment List ([list selection](#)) and click the [Align Selected Elements](#). Similarly, to generate a shared feature pharmacophore select two or more elements from the Alignment List and click [Merge Pharmacophores and Interpolate Overlapping Features](#). To remove elements from the Alignment List, perform a list selection on the desired elements and click [Delete](#) icon.

Show Advanced Options Check Box

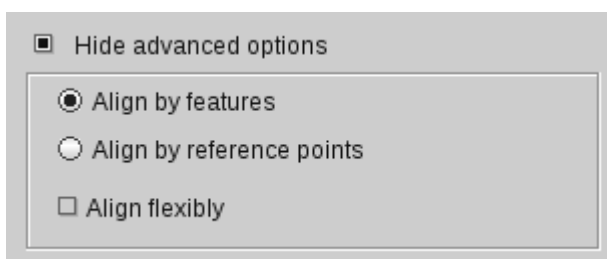


Figure 3.20. Advanced Options

If this check box is activated, LigandScout offers two pharmacophore and/or molecule alignment modes: The alignment based on [pharmacophore features](#), or on the [position of the respective protein residues \(reference points\)](#).

[Align by features](#) allows to align both ligands and pharmacophore models by an algorithm examining the best possible pharmacophoric feature overlay. If no reference element was specified one of the selected elements will be chosen randomly.

[Align by reference points](#) is a useful tool to investigate protein-ligand complexes of structurally related targets. It allows for aligning ligands with respect to the surrounding protein residues. In this way the user is able to directly compare the absolute position of one or several ligands within the aligned binding site.

If you want to include available conformations in the alignment process, enable the *Align flexibly* check box.

Alignment List

Molecules are represented by the [Molecule](#) icon and pharmacophores are represented by the [Pharmacophore](#) icon. Molecules and pharmacophores can be accessed (and selected for alignment) by clicking on their names in the Alignment List.

You can also select several elements next to each other by locating the mouse pointer on a single element, pressing the LEFT mouse button and dragging over the areas you wish to select. This *list selection* will be highlighted in the associated lines. To select an element click on the [Unselected](#) icon and to deselect this element again click on the [Selected](#) icon in the appropriate line. This special selection allows for a *coordinate-transforming rotation and translation* in the 3D Viewer: Select a ligand in the 3D View and press ALT+SHIFT+LEFT mouse button for rotating and ALT+SHIFT+RIGHT mouse button for translating (Command+Option on MAC). These special transformations can be used to manually align a structure to another one or to separate two aligned ligands for instance.

To toggle the visibility of an element click on the [Toggle Visibility](#) icon or to switch from blurred or de-saturated color representations to normal representations click on the [Context Representation](#) icon and to switch back click on the [Focused Representation](#) icon. The blurring feature is activated/deactivated with the [Alignment Perspective/Use Blur \(SDOF\)](#) command in the [Render Control](#) menu.

Context Menu

The *Context* menu extends the set of available user interactions by providing additional features for handling elements in the Alignment List. Select an element in the Alignment List and press the RIGHT mouse button to open the *Context* menu.

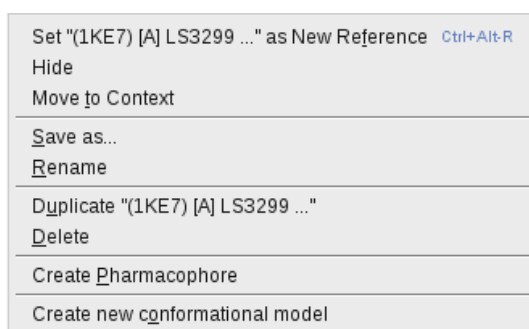


Figure 3.21. Context menu for alignment

Set "Element Name" As New Reference	This option will set the currently selected element as new reference element.
Hide/Show	Toggles visibility of the currently selected element.
Move to Context	Toggle the context state of the currently selected object.
Save as...	Save the currently selected object.
Rename	Rename the currently selected object.
Duplicate "Element Name"	Duplicate the currently selected element and add the duplicated element to the list.
Delete	Delete the currently selected element.
Create Pharmacophore	Creates a pharmacophore of the currently selected ligand(s). LigandScout thereby places all common pharmacophoric features on the molecule(s).
Create new conformational model	Creates conformations to the selected molecule.

Slider and RMS Label

The slider and the RMS label will stay inactive if an alignment was performed for three or more elements or if there was a perfect alignment (which is the case for a molecule aligned to its pharmacophore or for two instances of the same structure). If there were exactly two elements to be aligned which did not show a perfect alignment, the slider as well as the RMS label will become active and will give more information of the currently shown alignment. Use the slider to iterate over the alignments. The alignments are sorted according to a score, the best ones will be at the beginning, whereas values reflecting poor alignments will be at the end of the slider range. The RMS label gives the RMS (root mean squared) of the position deviances of all valid feature matchings. The number in brackets represents the number of valid feature matchings.

Render Options

To use the render options, select in the menu bar *Render Control > Alignment Perspective Defaults*. Use *Blur (SDOF: Semantic Depth of Field)* allows advanced blurring of objects. The effect is only visible if the [corresponding check box](#) of ligands/pharmacophores in the Alignment Panel is activated. Blurring may cause graphical issues or slow down the performance on low end computer systems and is therefore recommended only with suitable hardware equipment. *Show Reference Points* allows to visualize the position of the protein residues when using [alignments based on reference points](#). *Show Reference Point Names* places residue identifiers to this reference points. *Show Feature Tethers* visualizes the affiliation of corresponding pharmacophoric features with tethers. *Show Reference Point Tethers* visualizes the affiliation of corresponding protein reference points with tethers.

3.5. Screening Perspective

Searching in a large molecule collection for molecules possessing a specific pharmacophoric pattern is the next step in drug design. LigandScout's Screening Perspective provides the possibility to screen large molecule databases against 3D pharmacophore models.

The Screening Perspective consists of a special control panel, the Screening Panel and the Library View.

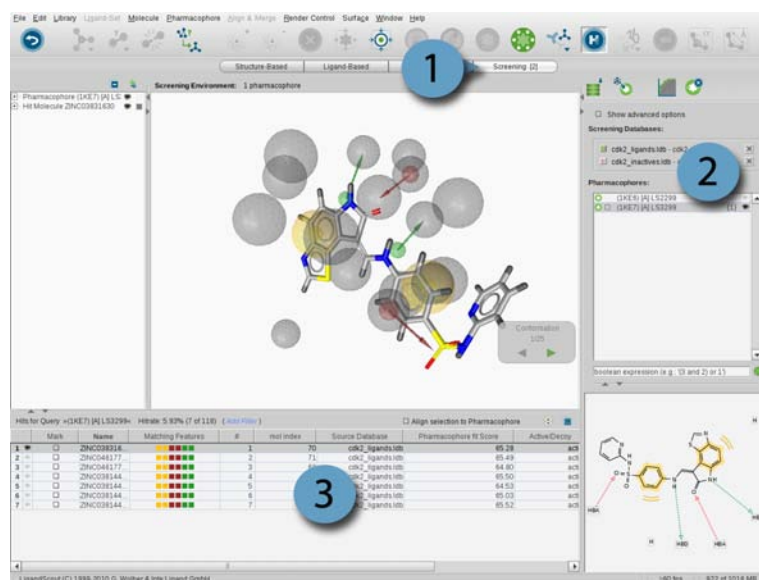


Figure 3.22. Screening Perspective (1), Screening Panel (2) and Library View (3)

3.5.1. Screening Panel

The *Screening Panel* allows you to manage your molecule databases and pharmacophores. It contains an icon toolbar, the *Show Advanced Options Check Box*, the Molecule Database List and a Pharmacophore List.

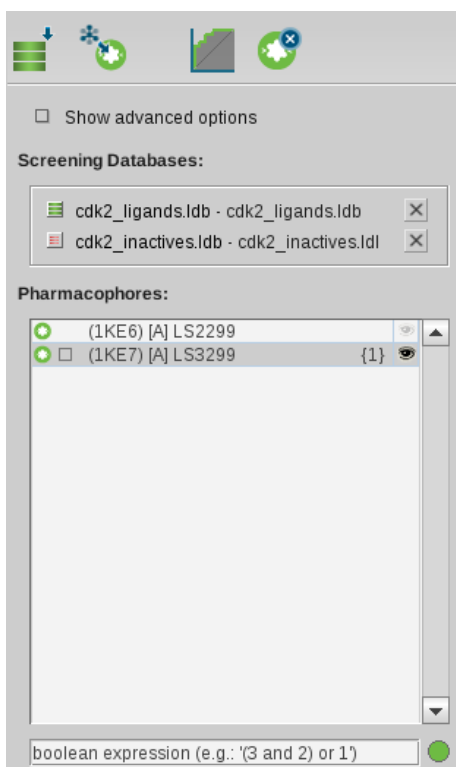


Figure 3.23. Screening Panel

Icon Toolbar

Use the [Load Database](#) icon to import a database (*.ldb) into the Molecule Database List or use the menu *File > Open*. A click on the [Perform Screening](#) icon starts the screening process. To show the ROC curve (receiver operating characteristic) press the [Plot ROC Curve](#) icon. If the screening process has not started yet, the screening starts automatically and after that the ROC curve is shown. Pharmacophores can be removed from the Pharmacophore List by selecting them in the Pharmacophore List and then pressing the [Delete Pharmacophore](#) icon.

Show Advanced Options Check Box

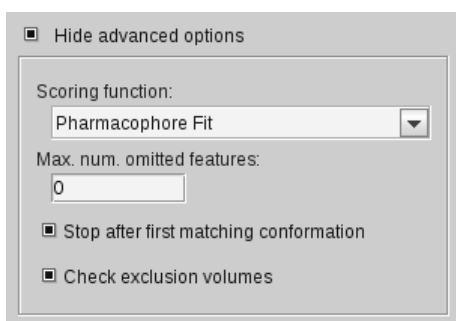


Figure 3.24. Show advanced options settings

To activate *Show Advanced Options*, click on the check box. You can choose the *scoring function* (default: Pharmacophore Fit), how many features of the pharmacophore should be omitted, stopping

after first matching conformation and if excluded volume clashes should be checked during the screening process. Another precondition for a hit is that the molecule has to match a minimum of three features found in the pharmacophore model! You can change these settings in the [Library Screening Settings](#) in the Preferences.

Molecule Database List

After importing a database through the [Load Database](#) icon or the *File > Open*, the database is added to the Molecule Database List. For loading time reasons, the molecules in the databases are not shown in the 3D View. To include a database, select the check box to the left of the appropriate database name. The check box will be marked green which assigns the database as active. Clicking again on this check box will mark this check box red, which symbolizes that the database includes inactive compounds. You can select more than one database but at least one needs to be marked as active. To remove a database select the cross button to the right of your database in the list. If you want to see the molecules in a database, simply switch to the Structure-Based Modeling Perspective and open the database.

Pharmacophore List

LigandScout allows one to screen a multitude of pharmacophores against multiple databases. Import pharmacophores into the Pharmacophore List through the menu *File > Open*. Optionally, you can copy them from other perspectives to the Screening Perspective by the [Copyboard Widget](#). For the screening process, make sure that at least one pharmacophore is selected and visible in the Pharmacophore List. To remove a pharmacophore, select it and press the [Delete Pharmacophore](#) icon.

LigandScout provides the functionality to combine pharmacophores using boolean expressions ("AND"/"OR"). If you select more than one pharmacophore, the pharmacophores are consecutively numbered. Define the combination of your pharmacophores in the query text box under the Pharmacophore List. Setting the pharmacophore numbers in brackets groups the expressions and ensures the order. The boolean expression will be automatically checked due to its operability. A red spot shows an error in the expression. The green spot signalizes that the expression is ready to use. For instance: "(3 AND 2) OR 1" means that a valid hit needs to be matched to pharmacophore 3 and 2, or only to pharmacophore 1. If nothing is specified in the query text box, the selected pharmacophores are connected by the "OR" expression.

3.5.2. Library View

After the screening process has finished, only the matched molecules (so called hit results) appear in the *Library View*. Information such as, name, number of conformations, and geometric fit are shown per molecule in the hit Library View table. In the Library View you can select the molecules one by one for further investigation, filter or sort the table entries. You may also calculate additional properties for your hit molecules by selecting the appropriate entry of the *Library* menu. For further information about the navigation in the Library View, please see [Section 3.1.5, "Library View"](#).

Chapter 4. Introduction to Pharmacophore Modeling

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This chapter provides general information about pharmacophore concepts.

1. [Pharmacophore Modeling](#)
2. [Pharmacophore Feature Definitions](#)
3. [Editing a Pharmacophore Model](#)

4.1. Pharmacophore Modeling

4.1.1. Pharmacophore Definition

What is a Pharmacophore?

A *pharmacophore* is defined as an ensemble of universal chemical features that characterizes a specific mode of action of a ligand in the active site of the macromolecule in 3D space. Chemical features are, e.g. hydrogen bonds, charge interactions and hydrophobic areas. This pharmacophoric pattern is the condition for ligand-macromolecule interaction. Searching these chemical patterns in large molecule databases allows you to find new scaffolds for developing lead structures.

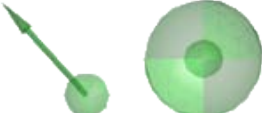








3D Pharmacophore Models in LigandScout

LigandScout generates structure-based as well as ligand-based pharmacophore models. The advanced 3D pharmacophores supports multiple features per heavy atom to broaden the scope of a single model. This approach enables you to have two hydrogen bond acceptors and one donor feature on the same

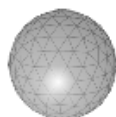
atom. LigandScout's integrated in-silico screening considers these multiple features locations. However, not all external in-silico screening applications support multiple features located on one heavy atom. If you are using the software-packages Catalyst, MOE or Phase, it is recommended to use the [Create Simplified Pharmacophore](#) routines.

4.2. Pharmacophore Feature Definitions

LigandScout provides the following pharmacophore features for automated pharmacophore generation:

Depiction in LigandScout	Pharmacophore Feature
	Hydrogen Bond Donor
	Hydrogen Bond Acceptor
	Positive Ionizable Area
	Negative Ionizable Area
	Hydrophobic Interactions
	Aromatic Ring
	Iron Binding Location
	Zinc Binding Location
	Magnesium Binding Location

Depiction in LigandScout



Pharmacophore Feature

Excluded Volume

While hydrogen bond features are defined by direction and distance constraints, hydrophobic interactions and ionizable areas have a distance constraint only. Dedicated features are supplied for the characterization of the binding of ligands to magnesium, zinc and iron. In addition to LigandScout's supported chemical features, the program increases selectivity of the generated pharmacophores with the *Excluded Volume Feature*. During pharmacophore generation, LigandScout analyzes the shape of the active site and places *excluded volume spheres* in positions that are sterically claimed by the macromolecular environment. This process ensures that molecules retrieved via virtual screening match the sterical requirements of the active site while simultaneously drastically increasing selectivity. [Excluded volume coats](#) are likely to further increase enrichment.

4.2.1. Pharmacophore Feature Constraint Types

Pharmacophore features are defined by following constraints types:

Distance Constraints

Distance Constraints represent the relation between two points, one located on the ligand side, one on the macromolecular side.

The following table shows LigandScout's default distance constraint settings:

Aromatic interaction with positive ionizable	3.5 - 5.5 Å
Aromatic interaction with ring (parallel)	2.8 - 4.5 Å
Aromatic interaction with ring (orthogonal)	2.8 - 4.5 Å
H-Bond interaction	2.2 - 3.8 Å
Hydrophobic interaction	1.0 - 5.9 Å
Iron binding location	1.3 - 3.5 Å
Magnesium binding location	1.5 - 3.8 Å
Negative ionizable interaction	1.5 - 5.5 Å
Positive ionizable interaction with negative ionizable	1.5 - 5.5 Å
Positive ionizable interaction with aromatic ring	1.0 - 10.0 Å
Zinc binding location	1.0 - 4.0 Å

Direction Constraints

Direction Constraints represent the relationship between two atom groups, one located on ligand side, one on macromolecular side. Groups form a rigid reference geometry, which are the basis for a directed vector.

4.2.2. Pharmacophore Feature Definitions

Hydrogen Bond Features

LigandScout discriminates flexible against rigid hydrogen bonds.

Rigid H-bond interactions, as occurring at sp^2 hybridized heavy atoms, are represented by a cone with cutoff apex. The default angle range for sp^2 hybridized heavy atoms is 50 degrees. See [Section 9.1.3, "Hydrogen Bonding"](#) for detailed instructions.

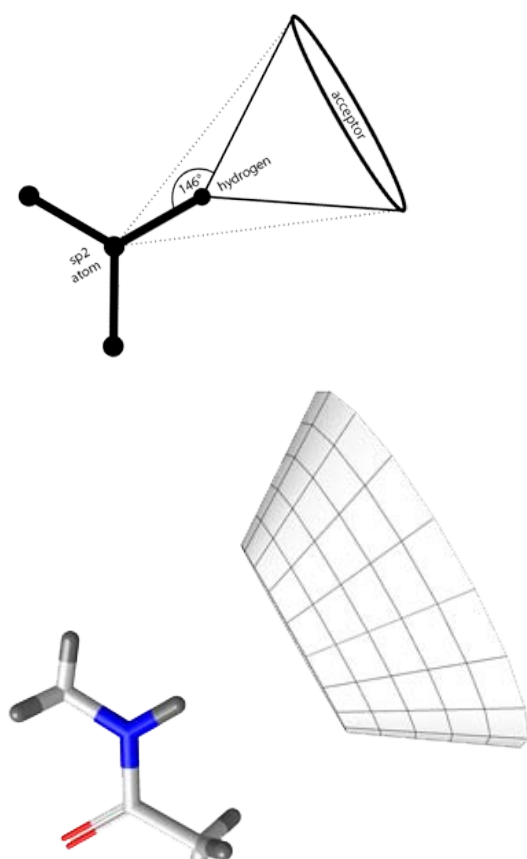


Figure 4.1. Rigid H-bonds constraint of an sp^2 hybridized amide nitrogen.

Flexible H-bond interactions, as occurring at sp^3 hybridized heavy atoms, are represented by a torus. The default angle range for sp^3 hybridized heavy atoms is 34 degrees. See [Section 9.1.3, "Hydrogen Bonding"](#) for detailed instructions.

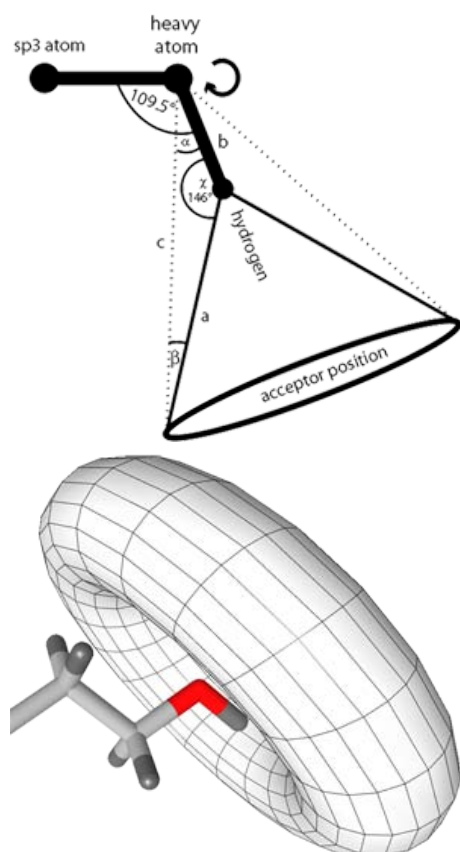


Figure 4.2. Flexible H-bonding constraint of an sp^3 hybridized hydroxy group.

Metal Binding Features (Magnesium, Zinc and Iron)

The maximum binding direction deviation for metal binding groups is 120 degrees; the respective binding symmetry deviation is 0.75 Å.

Charge Interaction Features

Charge interactions are considered to be distance constraints and are depicted as an astral center in LigandScout.

Hydrophobicity Features

Consistent with charge interaction features, also hydrophobicity features are regarded as distance constraints and are represented as a single sphere in LigandScout. Minimum thresholds and surface accessibility thresholds are user-adaptable. See [Section 9.1.5, “Hydrophobicity”](#) for detailed instructions.

Aromatic Features

Aromatic features represent pi-pi (orthogonal & parallel) and cation-pi interactions. LigandScout identifies aromatic systems of the ligand that are interacting with aromatics or cations in the environment. See [Section 9.1.6, “Aromatic Interactions”](#) for detailed instructions.

Excluded Volumes

Excluded volumes are derived from the sterical circumference of the macromolecule. LigandScout analyzes shape of the active site and places excluded volume spheres in positions that are sterically claimed by the macromolecular environment. Excluded volumes spheres are represented as single spheres in LigandScout. LigandScout's default settings are user-adaptable. See [Section 9.1.2, “Distance Ranges”](#) for detailed instructions.

4.3. Editing a Pharmacophore Model

4.3.1. Create New Features and Change Feature Types

LigandScout provides three geometric types of features: vector (H-Bond Acceptor, H-Bond Donor, Iron Binding Location, Magnesium Binding Location, Zinc Binding Location), point (Hydrophobic, Negative Ionizable, Positive Ionizable, Exclusion Volume) and plane feature (Aromatic Ring).

You can create a vector feature between the ligand and the environment by selecting an atom on ligand-side and on environment-side. Then, choose your appropriate feature in the menu *Pharmacophore > Create New Feature*. To create a feature only on the ligand-side, select at least one atom of the ligand. If you want to create an aromatic ring feature, at least three atoms are needed to be selected. If you choose more than one atom (ligand-side only), the new feature is created on the average position of the selected atoms. To switch the type of a feature, select a feature and go to the menu *Pharmacophore > Change Feature Type*. A submenu shows which types can be chosen.

4.3.2. Deleting Selected Features

LigandScout's automated pharmacophore generation algorithm is designed to create pharmacophores and [simplified pharmacophores](#) that are well-suited for most applications. However, you may want to manually delete features of a pharmacophore. Just select the features (spheres or vectors) in the [3D](#)

[View](#) and use the DEL key to erase the selected elements. The respective command *Edit > Delete Selected Elements* and the [Delete](#) button provide the same functionality.

Overlapping [hydrogen bond donor](#) and [acceptor features](#) are depicted as half-green and half-red vectors. Consistent with the respective feature type, LEFT mouse click on the green part selects the donor and on the red part selects the acceptor.

4.3.3. Deleting All Features at Once

The *Pharmacophore > Clear Pharmacophore* command allows you to delete all pharmacophore features at once. To reverse your actions use the [Undo](#) button or *Edit > Undo* command.

4.3.4. Increasing/Decreasing the Tolerance of Selected Features

The *Pharmacophore > Increase Selected Feature Tolerance by 0.15 Å* and *Decrease Selected Feature Tolerance by 0.15 Å*, respectively, allows you to adapt the tolerance spheres of selected features. The commands allow one to adapt the selectivity of pharmacophore models smoothly to individual needs. The tolerance spheres have high impact on the restrictivity of a model during virtual screening.

4.3.5. Edit Feature Weight

The commands in the menu *Pharmacophore > Increase Selected Feature Weight by 0.1* and *Decrease Selected Feature Weight by 0.1* changes the weight of specific features. The feature weight has an impact on the pair-wise alignment of pharmacophores. The value range is between 0 and 1, whereas a feature weight near to 1 has more importance. The small sphere in the origin of a feature changes its size according to the feature weight.

4.3.6. Marking Features

Mark Features as Optional flags the selected features which are not mandatory to find a valid alignment (similar to the max. omitted feature action). During the screening process, a molecule is still a valid hit when these marked features are not matched. *Unmark Features as Optional* reverses the flag of the selected features. To switch off features, select *Mark Features as Disabled*. These features are not taken into account in the alignment process. To switch disabled features on, select *Mark Features as Enabled*.

Chapter 5. Structure-Based Pharmacophore Design

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5.1. Structure-Based Pharmacophores

5.1.1. Why Use Structure-Based Pharmacophores?

Pharmacophores represent chemical functions, valid not only for the currently bound, but also unknown molecules. Due to its simplicity, this method is computationally very efficient and is subsequently exceptionally well suited for virtual screening of large compound libraries. Pharmacophore modeling is universal, comprehensive and editable: Selectivity-tuning by adding or omitting feature constraints provides a plenitude of manipulation options.

The following figures show the active site of CDK2 with ligand LS2 (PDB 1KE6) and the respective pharmacophore model generated by LigandScout. The program automatically searches for interactions between ligands and the macromolecule.

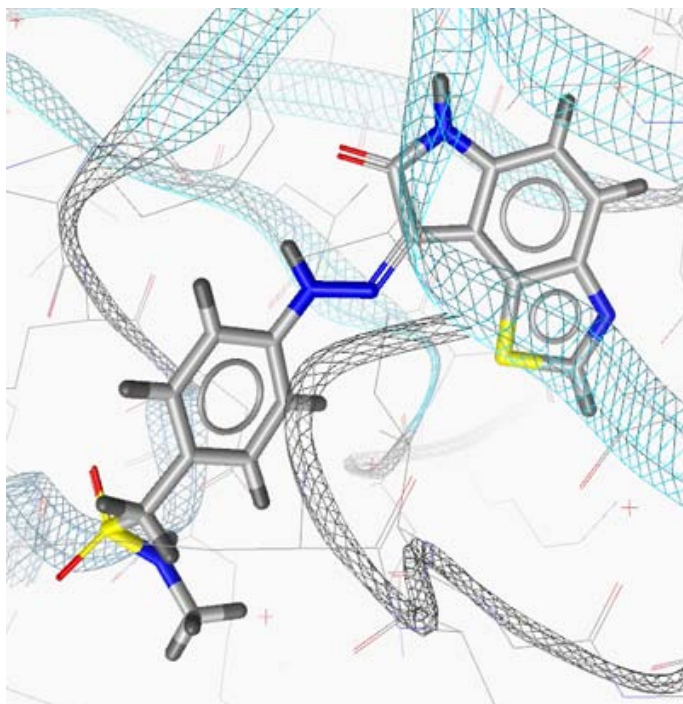


Figure 5.1. Ligand LS2 of PDB entry 1KE6 in the active site

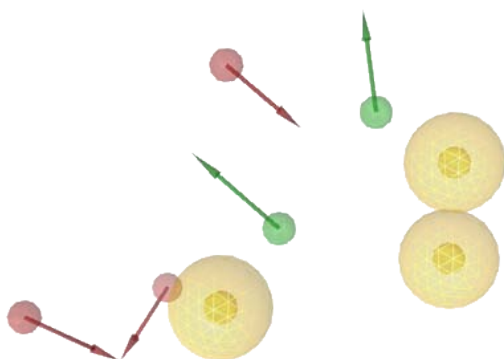


Figure 5.2. Structure-based pharmacophore model generated by LigandScout

In this example, LigandScout suggests an eight feature pharmacophore model consisting of three hydrophobic features (yellow spheres), two hydrogen bond donor features (green vectors) and three hydrogen bond acceptors (red vectors). This pharmacophore model represents a highly potent filter for in-silico screening of new CDK2 ligands.

5.1.2. Basic Workflow

The basic workflow for structure-based pharmacophore model generation based on PDB complexes consists of three main steps:

1. Extraction, identification, and interpretation of the ligand (hybridization states, bonds)

2. Deduction of the pharmacophore
3. Visualization, user interaction, and export for virtual screening

5.2. Creating Structure-Based Pharmacophores

5.2.1. Data Import and Structure Preparation

For the automated generation of pharmacophore models, please [import](#) your favored PDB complex to the Structure-Based Modeling Perspective in LigandScout. Since PDB data does not provide information on atom hybridization states and bond types, LigandScout uses a sophisticated ligand interpretation algorithm for the assignment of bond types via molecular geometry analysis. Data quality of PDB ligands is known to be an issue in molecular modeling. Therefore, manual verification of the automatically deduced ligand structure is highly recommended. Moreover, it represents a vital verification step for the subsequent pharmacophore generation process. Special care should be taken on double bond types and also on tautomerism. *Tautomers* are not discriminated by geometric analysis but have a decisive impact e.g. on hydrogen bond features.

As you have imported and verified your macromolecule-ligand structure, you are ready to run LigandScout's automated pharmacophore generation procedure. Please note that structure-based pharmacophore generation is only available in [Active Site View](#).

5.2.2. Automated Generation of Structure-Based Pharmacophores

In [Active Site View](#), simply press F9 on your keyboard to start the analysis. Alternatively, you may also use the [Create Pharmacophore](#) button or through the menu *Pharmacophore > Create Pharmacophore*. LigandScout will calculate the pharmacophore model within seconds.

LigandScout is designed for the retrieval of automatically created advanced 3D pharmacophore models supporting multiple features per heavy atom to broaden the scope of a single model. However, not all external in-silico screening applications support the wealth of pharmacophore feature handling which is incorporated into LigandScout.

If you are using the software-packages Catalyst, MOE or Phase it is recommended to use the [Create Simplified Pharmacophore](#) routines to achieve best compatibility to these external software applications.

You may also edit advanced pharmacophores for analysis using Catalyst, MOE or Phase by manually deleting features. In this case, it is recommended to first delete features interacting with water molecules and then to erase features that have unfavorable distances and angles. Try to obtain the best feature(s) for every heavy atom. You can use literature to prioritize interactions. Tutorials on pharmacophore preparation for [Catalyst](#), [MOE](#), and [Phase](#) provide more detailed information on this issue.

Please note that simplified pharmacophores are less restrictive and do not represent LigandScout's full capacity of pharmacophore generation.

5.2.3. Creating Advanced Structure-Based Pharmacophore Models

LigandScout supports several additional pharmacophore generation modes. More detail is provided [here](#).

5.2.4. Creating Excluded Volumes Coat

LigandScout supports the generation of an excluded volume coat for structure-based pharmacophore models via the menu *Pharmacophore > Create Excluded Volumes Coat*. Especially in the case of tight binding pockets, this approach allows one to significantly enhance the selectivity of the model. However, it is also well-suited for the simulation of binding cavities on the protein surface.

5.2.5. Creating Pharmacophore Models from Injected Ligands

Ligands can be injected into different context: binding pocket, solely ligand or pharmacophore. In the [Active Site View](#) you can add ligands from external sources by inserting a file containing molecules by the menu *File > Insert*. The external ligands have to be in the correct coordinate frame to yield a meaningful result. For instance, if the molecule to be injected was docked into the active site before, this restriction will be fulfilled. If the coordinate frame was not correct, the inserted ligand will be placed into the coordinate frame of the active site no matter if it makes sense or not.

In this way you can create pharmacophore models based on a certain docking pose for rapid virtual screening of novel leads. Another application of this feature is the investigation of docking poses, since it allows you to simply visualize the protein-ligand interactions of these conformers by generating the pharmacophore model.

If the external ligands are not in the coordinate frame, you can align them into the context. For more information on that, please see [Section 3.1.5, "Library View"](#) which enables you to navigate quickly through large compound collections.

Chapter 6. Pharmacophore Alignment

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6.3.1. Automatically Merge Pharmacophores	66
6.3.2. Manually Merge Pharmacophores	67
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LigandScout supports alignments of pharmacophores and molecules in arbitrary combinations. The generation of shared feature pharmacophores is a powerful tool to combine the knowledge gained from multiple pharmacophores.

1. [Aligning Pharmacophores and Molecules](#)
2. [Shared Feature Pharmacophore Generation](#)
3. [Advanced Pharmacophore Editing: Merged Feature Pharmacophore Generation](#)

6.1. Aligning Pharmacophores and Molecules

6.1.1. Aligning Pharmacophores and Molecules by Features

General

LigandScout supports feature-based alignments of pharmacophores and molecules in arbitrary combinations. Alignments are always performed pairwise no matter how many elements are to be aligned. Therefore, it is necessary to mark one structure as a reference element. In case the user did not specify a reference element explicitly, LigandScout will automatically choose one element from the set of selected elements. All other selected elements will be aligned to this *reference element*.

A single alignment is based on matched chemical features (feature pairs) where one feature must be the reference element's feature set and the other must be from the feature set of the element to be aligned to the reference element. For pharmacophores these chemical features are given explicitly. However,

for molecules these features have to be derived during the alignment algorithm and for the sake of clarity these are not visible to the user.

There are various ways to adjust the alignment algorithm. Please have a look at [Section 9.5, "Alignment Settings"](#) for details. We refer to the [Copyboard Widget](#) for more information on how to add pharmacophores and molecules to the Alignment List. Possible interactions in the Alignment Perspective are discussed in the [Section 3.4.1, "Alignment Panel"](#).

Performing Alignments

Setting the Reference Element (Optional)

You can set a reference element prior to perform the alignment by selecting one element in the Alignment List and clicking the [Set Reference](#) icon. Please note that setting a reference element is to be seen as a hint to the alignment algorithm. If the reference element was set and the reference element is also part of the selected elements to be aligned, this reference element will be used during alignment. In all other cases (no reference element set, reference element set but not contained in the selected elements) an arbitrary reference element will be chosen automatically.

Aligning Selected Elements

All elements which will be selected in the Alignment List will be aligned to a reference element. This reference element can be set by the user or will be chosen automatically as described above. To perform an alignment, select two or more elements in the Alignment List. This should look like the following image where pharmacophores of ligands of complexes 1ke5 and 1ke6 were selected and 1ke6 was set to be the reference element.

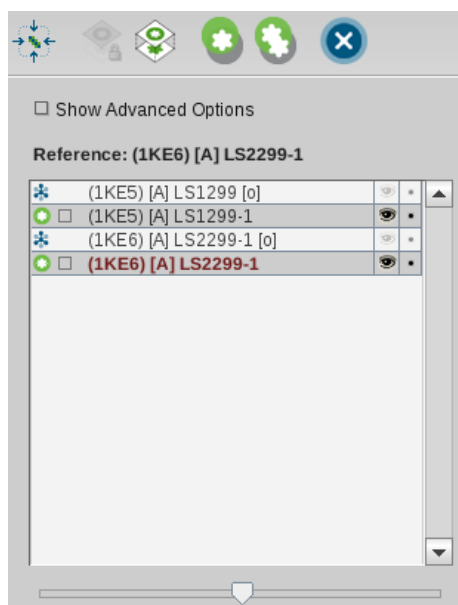


Figure 6.1. Before Alignment

After clicking the icon [Align Selected Elements](#) LigandScout will calculate one or several alignments and present them to the user as illustrated in the image below. The activated slider indicates that there are several alignments available. Information about each alignment are found in the *Alignment Details*

View, e.g., the associated RMS (root mean squared) value of the valid matched feature deviances is shown as well as the number of valid matched features.

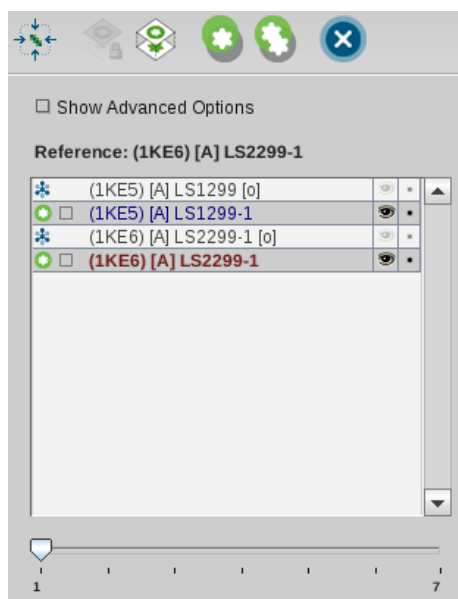


Figure 6.2. After Alignment

There are cases where LigandScout will provide just a single alignment:

1. Three or more elements will be aligned to each other
2. A perfect alignment was found (usually a molecule to its own pharmacophore, or aligning two instances of the same structure)

6.1.2. Aligning Pharmacophores and Molecules by Reference Points

General

LigandScout supports alignments of pharmacophores and molecules with respect to the protein environment. This is a useful feature to investigate protein-ligand complexes of structurally related targets but also to generate shared feature pharmacophores. In this way the user is able to directly compare the absolute position of one or several ligands within the aligned binding site. For this approach, the investigated proteins structures have to show a certain degree of similarity, in order to achieve a meaningful structural alignment.

Performing Alignments

First, check the *Show Advanced Options Box* in the *Alignment Perspective*. LigandScout now offers both alignment modes: The *Feature-Based Alignment* mode and the *Reference Points-Based Alignment*

mode. Activate the latter one and select the pharmacophore and/or molecules you want to align. Clicking onto the icon [Align Selected Elements](#) calculates the alignment of these elements with respect to their protein environment. In other words, LigandScout aligns the protein residues (which is by default not visible in the Alignment Perspective) of all selected elements in order to overlay the pharmacophores and molecules according to their position in the binding sites.

You can visualize the protein alignment by toggling on the reference points in the Hierarchy View or select in the menu *Render Control > Show Reference Points* in the Alignment Perspective. The protein residues will thereby be illustrated as small circles. In the same menu, *Render Control > Show Reference Point Names* allows you to place the residue identifiers on these reference points. *Show Reference Point Tethers* visualizes the affiliation of corresponding protein reference points with tethers.

6.2. Shared Feature Pharmacophore

The generation of shared feature pharmacophores is a powerful tool to combine the knowledge gained from multiple pharmacophores. LigandScout creates a shared feature pharmacophore using a set of selected elements and aligns them using the alignment algorithm. The resulting alignment of all selected elements will be searched for valid feature overlappings. This means that one feature of a fixed element is considered and all its valid matchings during the alignment. If this feature was validly matched by one feature of each of the other elements these features are considered as a valid matched feature set. If every feature lies within the tolerance of all the other features in this valid matched feature set, a new feature will be derived by combining all the original features. The resulting feature consists of the mean of all positions and the mean of all tolerances. This combination is done for every set of overlapping features and results in a shared feature pharmacophore.

6.2.1. Creating a Shared Feature Pharmacophore

To perform a shared feature pharmacophore generation select two or more elements from the Alignment List and click on the icon [Generate Shared Feature Pharmacophore](#). LigandScout will calculate alignments and valid feature overlappings and will combine all valid feature overlappings into a shared feature pharmacophore which will be presented after the algorithm has finished successfully.

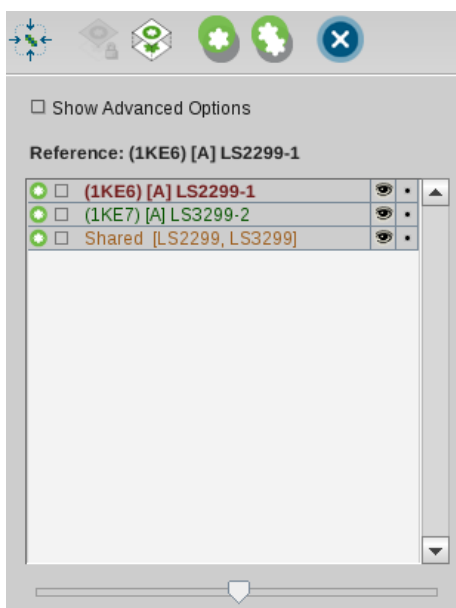


Figure 6.3. After Shared Feature Pharmacophore Generation

6.3. Merged Feature Pharmacophore

In case you would like to have more control over the generation of shared feature pharmacophores you can use LigandScout's ability to edit existing pharmacophores in the [Alignment Panel](#). This includes aligning pharmacophores and molecules by chemical features, merging these pharmacophores, and interpolating their features to reduce overlaps. These editing functionalities can be either performed fully automatically or manually.

6.3.1. Automatically Merge Pharmacophores

The fully automatic way to merge pharmacophores involves first aligning all of the selected elements, merging all features into a single pharmacophore and interpolating features which overlap too much. To perform this action select two or more elements from the Alignment List and click on the icon [Generate Shared Feature Pharmacophore](#). LigandScout will then calculate several alignments. If there was no valid alignment an error message will be displayed. Otherwise, LigandScout will choose the best alignment and use it for merging the pharmacophores of the selected elements. Finally, features which overlap too much will be combined into a single feature. After the algorithm has finished successfully, the aligned, merged, and cleaned-up pharmacophore will be presented in the Alignment List and the 2D/3D Viewer.

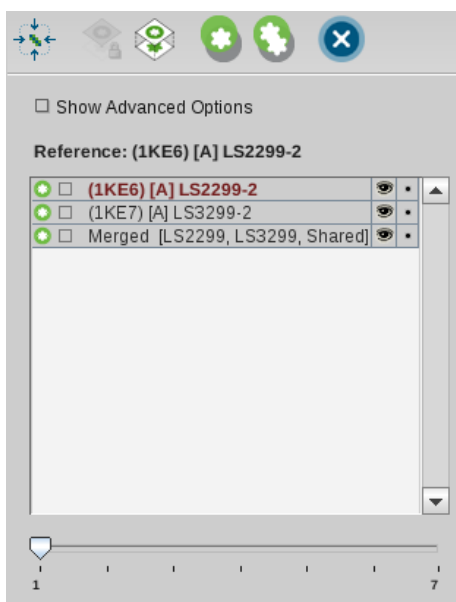


Figure 6.4. After merging pharmacophores

6.3.2. Manually Merge Pharmacophores

Align

You can use the automatic alignment as described in [Aligning Pharmacophores and Molecules](#) or you can align several structures manually by selecting an element in the 3D Viewer, pressing Alt+Shift and using LEFT or RIGHT mouse button to change positions of this selected element relative to the other elements.

Merge Pharmacophores

Two or more pharmacophores can be merged by selecting two or more elements in the Alignment List and pressing CTRL+ALT+M (Option+Command+M on MAC). The resulting pharmacophore will contain a copy of every feature from the selected pharmacophores or the pharmacophores of the selected molecules. The resulting pharmacophore can be cleaned up from overlaps automatically (see below).

Interpolate All Features Within Tolerance

Select one pharmacophore in the Alignment List and press CTRL+ALT+T (Option+Command+T on MAC). This will trigger LigandScout to detect and group overlapping features automatically. Each group of features will be replaced by the interpolated feature. So the resulting pharmacophore will contain minimal overlap.

Interpolate Features

You can also select features of the same type and from the same pharmacophore in the 3D Viewer and have them interpolated by pressing CTRL+E (Command+E on MAC). Here, the restrictions are not as tight as in the automatic way. You can select features within the range of five times the average tolerance of the selected features. If all possible pairs formed from the selected features are within this range this interpolation will be performed. The previously selected features will be replaced by the interpolated ones.

6.3.3. Combine Features in One Pharmacophore

LigandScout supports the combination of all features of several aligned pharmacophores to one model. This is useful, e.g., if there are two or protein-ligand complexes available and you want to combine the interactions formed by these ligands. Use the *Pharmacophore > Combine Features in One Pharmacophore* command to perform the automated combination of the currently selected pharmacophores.

In most cases you may want to downsize the number of features of the new model, especially, if there are features of the same type adjacent to each other. Therefore, you can either use the *Pharmacophore > Interpolate All Features Within Tolerances* command for a global merge, or you manually select the features (of the same feature type) that you want to interpolate and use the *Pharmacophore > Interpolate Features* command for manual merging (see above).

Chapter 7. Ligand-Based Pharmacophore Design

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7.1. Ligand-Based Pharmacophores

7.1.1. Why Use Ligand-Based Pharmacophores?

The ligand-based strategy deals with the absence of the macromolecule structure by deriving pharmacophore models from a set of ligands. This approach considers the conformational flexibility of the ligands.

Similar small molecules provide similar biological activity. Thus, this approach searches for a common feature pattern that is shared in an active ligand-set. The following figures show the ligands (LS2, LS3 and LS4) of 1ke6, 1ke7 and 1ke8 which bind to CDK2. LigandScout automatically creates the respective pharmacophore to these ligands.

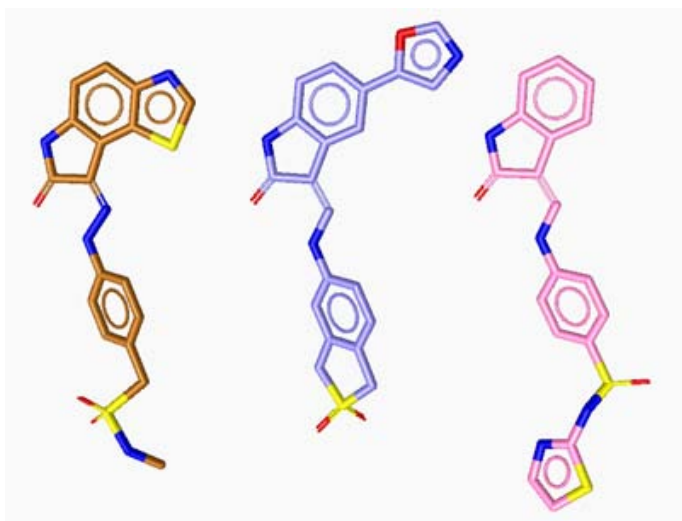


Figure 7.1. The ligands LS2 (brown), LS3 (blue) and LS4 (pink) of the PDB entries 1ke6, 1ke7 and 1ke8

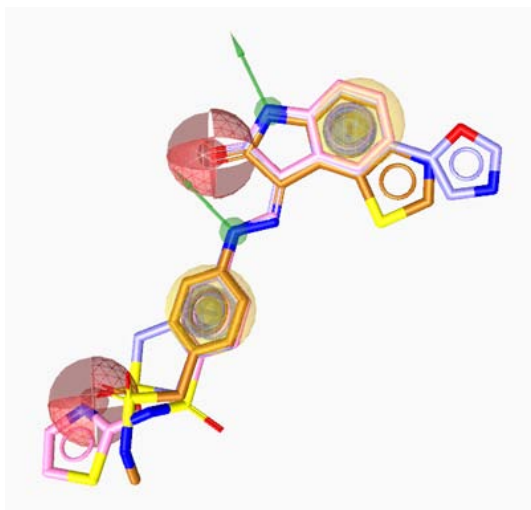


Figure 7.2. LigandScout's ligand-based pharmacophore model aligned with LS2, LS3 and LS4

In this case, LigandScout generates a ligand-based pharmacophore which is composed of two hydrophobic features (yellow spheres), two aromatic rings (grey elements), two hydrogen bond donor features (green vectors) and two hydrogen bond acceptors (red vectors).

7.1.2. Basic Workflow

The workflow for ligand-based pharmacophore model generation based on a set of ligands is as follows:

1. Define and prepare Ligand-Set (Training-Set, Test-Set)
2. Ligand-based pharmacophore creation
3. Visualization, user interaction, and export for virtual screening

7.2. Creating Ligand-Based Pharmacophores

Ligands which bind to one specific target protein, possess a similar binding mode. The goal of the ligand-based pharmacophore modeling is to seek common features in a set of ligands. LigandScout provides an automated generation of ligand-based pharmacophore models in the [Ligand-Based Modeling Perspective](#) or [command line](#).

7.2.1. Define and prepare Ligand-Set

A vast literature search of your target protein and its binding ligands are required. Although, if there are no protein structures available, the information about essential amino acids for the binding mode gives hints for verification of the results afterwards.

Define Ligand-Set

Gather known active ligands (at least 2) that bind to your target protein. This group of ligands serves as a Training-Set for the main pharmacophore generation process. These molecules are overlapped according to their pharmacophoric features and then several pharmacophore models are created that are found in all Training-Set molecules. A similar feature scaffold is one indication for this group.

For instance, you can load in the Structure-Based Modeling Perspective 1ke6, 1ke7 and 1ke8 one by one and add the ligands to the [Ligand-Based Modeling Perspective](#) by the [Copyboard Widget](#). In case you have an externally prepared ligands or Ligand-Set, just load it into the Ligand-Based Modeling Perspective by the menu *File > Open* or alternatively, use the *Ligand-Set > Add Molecules: To Training-Set/Test-Set*.

Optionally, you can prepare a Test-Set, that includes actives and inactives. This set is used for the verifying step, where the resulting pharmacophores are aligned to the Test-Set molecules. Ligands which you do not want to include in the process, can be put to the Ignored Ligands set. The type of the ligands can be changed through the menu *Ligand-Set > Flag Molecule As* or switching the type in the Ligand-Set Table.

If you are not sure how to divide your ligands into Test-Set and Training-Set, then LigandScout's clustering method assists your choice. The aim of this clustering is to choose those compounds that are similar in terms of 3D pharmacophore characteristics and therefore bear a higher chance for delivering a large overlap of chemical features. The 3D clustering algorithm performs fast alignments and clusters based on a similarity value between 0 and 1. Since this algorithm basically performs combinatorial alignments of all conformations of all compounds, a low number of conformations (1-3) is recommended. The cluster distance can be varied until the desired cluster size has been reached. To use the clustering method, select the [Clustering](#) icon. Adapt the settings for clustering and conformation generation (if no conformers are available yet). Then, click the OK button to run the clustering process. A new column *Cluster ID* appears in the [Ligand-Set Table](#). The values show the group which the ligands are assigned to. In the Ligand-Set Table, go through the ligands of each group to decide which type of set you want to use for each ligand.

Generate Conformations

To generate conformations for your ligands press the [Generate Conformations for Ligand-Set](#) icon. A dialog appears where you can customize the [settings](#). Alternatively, you can generate conformations through the [command line](#).

7.2.2. Performing Ligand-Based Pharmacophore Creation

Ligand-Based Pharmacophore Creation in LigandScout

When the ligand-set is ready to use for the pharmacophore generation, just press the [Run Ligand-Based Pharmacophore Creation](#) icon. A [settings](#) dialog appears where you can adjust the properties for the process.

To understand how the settings influence the pharmacophore creation, the process is explained shortly. First, conformations of the Training-Set molecules are generated (if not available). After ranking the molecules according to their number of conformations (flexibility), pharmacophore features (lipophilic points, hydrogen bond donors and acceptors, positive and negative ionizable groups) are projected on these molecules and all their conformations. All conformations of the two top ranked (i.e. the least flexible) molecules are then aligned using Inte:Ligand's molecular alignment algorithm. For a configurable number of best alignment solutions ("intermediate alignment solutions") common pharmacophoric features are interpolated and intermediate pharmacophore models are created and stored for further processing ("intermediate pharmacophore models"). These intermediate pharmacophore models are now ranked using several adjustable scoring functions taking into account chemical feature overlap, steric overlap, or both. The intermediate pharmacophore models are then aligned to all conformations of the third molecule, etc., and a new set of intermediate combined feature pharmacophores is created until all molecules have been processed. If at any stage no conformation can be found that can be matched on any intermediate solution, the process is stopped. If at least three common chemical features can be identified throughout the whole alignment and interpolation process, the feature pharmacophore combination is considered to be successful. All these steps can be logged in a file verbosely. The output can either be written in LigandScout's internal file format (PML or PMZ) or e.g. in Catalyst/Discovery Studio hypoedit format for use in Catalyst.

If the pharmacophore is exported to Catalyst, one must take into account that Catalyst is unable to map two features on one single ligand atom - in this case the Catalyst hypothesis can be reduced by deleting features or create several hypotheses for sequential virtual screening.

For forming intermediate solutions, two modes for sequential pairwise alignment are provided: shared and merged feature pharmacophore combination mode. While with shared feature combination mode an intersection between all pharmacophoric features of the underlying molecules is built (logical AND), with merged feature pharmacophore building mode, the features of the aligned molecules will be merged (logical OR). To avoid huge pharmacophores from merged feature pharmacophores, a parameter "number of omitted features" must be specified. This parameter defines how many features per molecule

may be omitted by the algorithm with respect to the final pharmacophore solution. Specifying a value of “1” for this parameter makes sure that a maximum of one feature is not matched by each of the Training-Set molecules.

If you want to generate exclusion volumes to the ligand-based pharmacophore model, just toggle on the *Create Excluded Volume* check box in the Ligand-Based Pharmacophore Creation dialog. To sterical limit the pharmacophore, excluded volumes are placed around the best alignment model.

For more information about the settings, please see [Section 9.2, “Ligand-Based Modeling Settings”](#) and [Section A.6, “Scoring Function”](#).

7.2.3. Resulting Ligand-based Pharmacophore Models

After the ligand-based pharmacophore generation is finished, the results are listed in the [Results Table](#). Select the first result in the table. In the Hierarchy View make all ligands visible by selecting the [Toggle Visibility](#) icon of each ligand. Thus, you can see how well the resulting pharmacophore fits to the ligands in the 3D View. In the Ligand-Set Table the *Feature Pattern* column shows the color encoding of the features, that matches the ligands. There, you can see which features cover the ligands well.

Chapter 8. Virtual Screening

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Virtual Screening (also called in-silico screening) is a computational approach to select molecules with desired properties in large molecule libraries. This technique is used to find new lead candidates in the drug development process. Furthermore, it can reduce the assay-to-lead attrition rate, time and expensive experiments during the process. LigandScout now allows you to in-silico screen molecule libraries using 3D pharmacophore models as filter criteria. Furthermore, LigandScout pharmacophores can be exported for virtual screening in other programs such as Catalyst, MOE and Phase.

8.1. Virtual Screening in LigandScout

Ligands with similar pharmacophoric patterns can exhibit similar biological activities according to one specific protein. Thus, this chapter gives a few guide lines how to model a pharmacophore and find ligands of a molecule library, that match to that specific pharmacophore. You can use the virtual screening tool in [LigandScout](#) or through the [command line](#).

8.1.1. Pharmacophore and Molecule Library Preparation

Create a pharmacophore model ([structure-based](#) or [ligand-based](#)), according to a target protein, that is capable to find a high amount of active ligands and as few as possible inactives. Here we refer a structure-based pharmacophore of 1ke7 complex as an example. One of the essential steps is an exhaustive literature search about your protein of interest. Where does the ligand bind? Which amino acids are required such that the ligand can interact with protein's binding pocket. Thus, this leads to the knowledge about features that should be contained in the pharmacophore. Which ligands bind to the protein and show biological activity and which do not? Load your protein-ligand complex in the [Structure-Based Modeling Perspective](#), zoom into the active site, check the double bonds of the ligand and create a pharmacophore. Check if the essential interactions are present and [edit](#) the pharmacophoric features if required.

It might be that several pdb complexes are available and therefore many pharmacophore models can be generated. In the [Alignment Perspective](#) you can edit the pharmacophores, align them, generate a shared or merged feature pharmacophore. Just add your pharmacophores and ligands one by one to the Alignment Perspective and investigate them until you have created an appropriate pharmacophore.

See also [Chapter 5, Structure-Based Pharmacophore Design](#), [Chapter 6, Pharmacophore Alignment](#) and [Chapter 7, Ligand-Based Pharmacophore Design](#) for further information on pharmacophore creation.

LigandScout uses the proprietary LDB file format for storing molecule libraries which helps to efficiently speed up the screening process. Hence, it is necessary to convert your molecule library into the LDB file format before you load your library. For instance, if you open a SDF file in LigandScout, you can export your library as a LDB file through *File > Save as File*. Alternatively, you can use the command line tool [ldbgen](#) to generate conformations and to convert your database into the LDB file format.

8.1.2. Run Virtual Screening

Add your pharmacophore model to the [Screening Perspective](#), e.g., by the [Copyboard Widget](#). Switch to the Screening Perspective and load a molecule library (.ldb) by clicking the [Load Database](#) button. Then, choose a pharmacophore. LigandScout offers you to select more than one database to be screened against the selected pharmacophore. Select and mark the database containing active ligands in green. If a decoy database is available that includes inactive ligands, mark it in red. To customize the advanced screening settings, toggle on the *Show Advanced Options Settings* in the [Screening Panel](#). Then, start the screening process by pressing the [Perform Screening](#) button.

Optionally, you can screen a pharmacophore against an external database in the Structure-Based Modeling Perspective. Make sure, that one pharmacophore is visible in the 3D View and select in the menu *Pharmacophore > Screen Pharmacophore Against External Library*. A [screening settings](#) dialog appears. Select your molecule database and modify the settings as required.

To execute the virtual screening process through the command line, please see [Section A.5.3, "Virtual Screening"](#) in the Appendix.

8.1.3. Screening Results

When the screening process is finished, a hit list of molecules, that matches the pharmacophore is shown in the [Library View](#). Click the results one by one and look how the ligands are aligned to the pharmacophore in the 3D View.

To see the ROC plot, press the [Plot ROC Curve](#) icon. The ROC curve shows how good the pharmacophore model discriminates between active and inactive ligands. The AUC value (area under the ROC curve) quantifies the ROC curve between 0 and 1. A value near to 1 is favorable. If you want to export the ROC plot as image, select in the menu *File > Save as File*. Choose an appropriate image file format

(e.g. PNG) and enter a file name in the Save as dialog. Then, select the ROC check box plot of the 3D export properties.

If the screening results produce less active hits, there can be several reasons. Question yourself, if the library ligands violate excluded volumes or is the pharmacophore too restrictive? Are the conformers of the ligands meaningful? Are essential features found in the ligands? To get more hits, [edit](#) your pharmacophore model. For instance, remove an excluded volume or other features or make the tolerance sphere larger and screen again. Another way is to raise the [number of maximum omitted features](#), e.g., 1 or 2, depending on the size of the pharmacophore or designate some features as optional. To do so, not all of the resulting ligand hits may contain all of the features of the pharmacophore model, but again these can lead to interesting results. If you have the problem that too many hits are found, then the model is too general and you should restrict your pharmacophore model. You can create [excluded volumes](#) or an [excluded volumes coat](#). An excluded volumes coat consists of many small excluded volumes which reflect the spatial geometry of the binding pocket. Note that the combination of generalizing and restricting the pharmacophore model is essential for influencing the hit results.

After all, finding a representative pharmacophore model of a ligand-protein binding mode needs a lot validation. One approach is to include active and inactive ligands in the molecule library. If most of the active ligands are contained in the hit results, then the pharmacophore covers the actives molecules well. On the other hand, if inactive molecules are matched, then the pharmacophore might possess the main feature scaffold matching the active ones; however pharmacophore model could not differentiate between the active and inactive ligands. In this case you will need to build an improved model.

8.2. Preparing a Pharmacophore for Virtual Screening with External Applications

LigandScout creates pharmacophore models that can be used in various external applications for virtual screening. The following tutorials will show you how to prepare your pharmacophores to be used in Catalyst, MOE, and Phase.

1. [Preparing a Pharmacophore for Virtual Screening with Catalyst](#)
2. [Preparing a Pharmacophore for Virtual Screening with MOE](#)
3. [Preparing a Pharmacophore for Virtual Screening with Phase](#)

8.2.1. Preparing a Pharmacophore for Virtual Screening with Catalyst

Creating a Catalyst compatible Pharmacophore

LigandScout is designed for the retrieval of automatically created [advanced 3D pharmacophore models](#) supporting multiple features per heavy atom to broaden the scope of a single model. However, not all in-silico screening applications support multiple features located on one heavy atom.

If you are using the software-package Catalyst, it is recommended to use the *Create Simplified Pharmacophore (Catalyst)* routine. The process is started by pressing SHIFT+F9 or using the *Create Simplified Pharmacophore (Catalyst)* command in the *Pharmacophore* menu. In this case, LigandScout achieves compatibility by searching for the best feature for every heavy atom according to a feature priority. This priority can be adjusted in the 'chemicalFeatureDefinitions.xml' file.

You may also reduce pharmacophores for Catalyst by manually deleting features. In this case, it is recommended to first delete features interacting with water molecules and then to erase features that have unfavorable distances and angles. Try to preserve the best feature for every heavy atom if there are multiple features on one heavy atom. You may also use literature to sort out interactions.

Exporting and Converting Pharmacophores for Use in Catalyst

Open the [Save](#) dialog by selecting *File > Save as File* in the menu bar. Choose *Pharmacophore as Catalyst Hypoedit Script (*.hypoedit)* in the file type drop-down menu, enter a file name and export the pharmacophore. It is highly recommended to also export the ligand as MDL Mol file for later hypothesis verification. Herewith, work in LigandScout is finished.

Next, the *.hypoedit* script has to be converted into a *.chm* hypothesis file, which can be imported into Catalyst as a hypothesis ready to be used for virtual screening.

The following steps assume that you have properly installed Catalyst on your workstation. Since LigandScout uses an extended dictionary, you have to provide this file, that is shipped with your LigandScout distribution, for the conversion step. As LigandScout sets the dictionary path in exported *.hypoedit* scripts to `$CATALYST_DICTIONARY/Dictionary.chm` you either have to overwrite your `$CATALYST_DICTIONARY/Dictionary.chm` with the LigandScout version or you use a text editor to adapt the second row of the *.hypoedit* file (i.e. `-dict $CATALYST_DICTIONARY/Dictionary.chm \`) with respect to the location of LigandScout's *Dictionary.chm* version.

Now that all environmental needs are accomplished, use the command `chmod a+x filename.hypoedit` to flag your script as executable. Run the script by typing `./filename.hypoedit`. The script starts a short conversion process resulting in a *.chm* file that you can import in Catalyst.

Importing a Hypothesis into Catalyst

Run Catalyst and import your *.chm* file by selecting in the menu *Data > Import*.

Verifying the Hypothesis in Catalyst

It is highly recommended to verify Catalyst's fitting capability of this hypothesis with the respective ligand. Only when Catalyst is able to fit ligand and hypothesis, can you be assured that Catalyst will find the respective ligand in databases. Thus, please also import the ligand's MDL Mol file into your stockroom. Drag & Drop both compounds into the *Hypotheses Workbench*. To open the *Show Conformational Model* dialog, select in the menu *Tools > Show Conformational Model*. Select *conf1* in the *Conformers Panel* and click on *Register Confs* at the bottom of the window. Now that this conformer is registered, return to your workbench and ensure that both ligand and hypothesis are still selected. To use the *Compare/Fit* function, choose in the menu *Tools > Compare/Fit*. A new window will pop up on the left side of your workbench offering several fitting options. For a first test, try to get a fitting with the defaults. Catalyst will instantly depict a fitting in most cases. If you are unable to retrieve a fitting, please see the *guidelines and suggestions* below for troubleshooting instructions. Otherwise, try to fit these compounds with *Max. Omitted Features* set to '0'. This modification causes Catalyst to consider the fitting of every single feature of the hypothesis. Fitting will only be successful, if all features can be fitted and only if this fitting is successful, Catalyst will find this ligand by database search. In other words, if you want to ensure that your ligand will be retrieved by Catalyst during a database search, you have to ensure that Catalyst is able to fit this compound in the *Hypotheses Workbench* with *Max. Omitted Features* set to '0'. The following list provides guidelines and suggestions on how to deal with the fitting issue.

Fitting Hypotheses in Catalyst: Guidelines and Suggestions

It may seem quite complicated to you to fit LigandScout hypotheses in Catalyst. However, with the following suggestions you will be able to get fittings of highly selective pharmacophores the quick and easy way!

1. Make sure that there are no multiple features on one heavy atom in the pharmacophore. Catalyst does not support multiple features on the same position in 3D.
2. Set *compare.minInterBlobDistance=0* in the *.Catalyst* parameters file to decrease the overall minimum distance between features. Please see the Catalyst documentation for detailed instructions on the *.Catalyst* parameters file
3. Set *FunctionMapping.Hydrophobe.Neighbor.numBondLessEqualFromOWithDouble-Bond=0* in the *.Catalyst* parameters file if you experience fitting problems of hydrophobic features e.g. with sulfonamides.
4. Try to get a fitting with *Max. Omitted Features* set to '1' first, and only if this works, with the parameter set to '0'
5. Locate the problematic pharmacophore feature(s): With *Max. Omitted Features* set to '1', analyze the fitting. Fitted features with projection point (e.g. hydrogen bond features) show a vector. This vector lacks in omitted features! In omitted spheres of all feature types the central, small, light-blue sphere is missing! Excluded volumes are not problematic for the fitting process and can be disregarded.
6. Hydrophobic features are most likely to be omitted by Catalyst's fitting algorithm. Special care should be taken on methyl moieties and chains.
7. Watch out for very tight feature distances, e.g., at heterocyclic systems. These moieties tend to have a lot of features focused in small space, which can be an issue.
8. In some cases, hydrogen bonds are only fitted after generation of a conformational model, because Catalyst is very restrictive on interaction angles. Generate conformers to check if the features get fitted. However, it is recommended to adjust pharmacophores to be fittable with the bioactive conformation of the PDB complex.

9. Hydrogen bond feature fitting problems are most likely to occur at heterocyclic and sterically hindered moieties.
10. Dismount the pharmacophore beginning with suspect features and always save intermediate steps as local object with *Data > Save As Local Object*.
11. Catalyst may deny fitting hydrogen bond features due to the location of its projected points. Try to delete this sphere and the respective vector. Even without direction, these features are valuable for highly selective pharmacophores.

Adding Features in Catalyst

In special cases you may want to add a feature to a pharmacophore within Catalyst.

Following guide shows you how to add features by using the *Show Function Mapping* feature, which enables accurate placement of new features on heavy atoms of the PDB ligand.

1. Import ligand and hypothesis to your workbench and prepare the pharmacophore in a way that Catalyst is able to achieve fitting with *Max. Omitted Features* set to '1'.
2. Add your favored feature (e.g. a hydrogen bond acceptor) to the desired heavy atom with Catalyst's *Show Function Mapping* feature located in the *Tools* menu.
3. Use LigandScout to retrieve the coordinates, e.g., for the projected point of your hydrogen bond acceptor feature. Click the [Toggle Tooltips](#) icon of the LigandScout tool bar. LigandScout shows you the exact position of the currently selected item in a box adjacent to your selection. These coordinates can be used to set the location constraints in Catalyst.
4. Select the respective projected point of the hydrogen bond feature in your *View Hypotheses* workbench and alter the coordinates in the *Set Constraint Tolerance* dialog. The Catalyst documentation gives you detailed information on how to add features to a hypothesis.

8.2.2. Preparing a Pharmacophore for Virtual Screening with MOE

Creating a MOE compatible Pharmacophore

LigandScout is designed for the retrieval of automatically created [advanced 3D pharmacophore models](#) to broaden the scope of a single model. However, not all in-silico screening applications support exactly the same features or define their features in exactly the same way.

If you are using the software-package MOE, it is highly recommended to use the *Create Simplified Pharmacophore (MOE)* routine. The process is started by pressing CTRL+SHIFT+F9 (Shift+Command+F9 on MAC) or selecting *Pharmacophore > Create Simplified Pharmacophore (MOE)*. In this case, LigandScout achieves compatibility by merging features at the same position and by adding charge features *if and only if* one of the underlying atoms have an explicit charge. Also the hydrogen bond lengths have to be adjusted to be 1.8 Å.

Exporting and Converting Pharmacophores for Use in MOE

Open the [Save as File](#) dialog in the *File* menu. Choose *Pharmacophore in CCG MOE Format (*.ph4)* in the file type drop-down menu, enter a file name and export the pharmacophore in one of the two supported MOE pharmacophore schemes. It is highly recommended to also export the ligand as MDL .mol file for later hypothesis verification.

Importing a Hypothesis into MOE

Run MOE and import your .ph4 pharmacophore by selecting in the menu *File > Open* . Choose your .ph4 file, select *moe_ph4* in the drop-down box next to *Force type* and click *OK*.

Verifying the Hypothesis in MOE

It is recommended to verify the exported pharmacophore against its originating molecule in MOE. To do this, you first create a database by selecting in the menu *File > New > Database...* . Enter a name for your database and click *OK*. Now you need to import your previously exported MDL .mol file into the newly created database. Select *Import > SD* from the *File* menu. This will open a new dialog. Click on *Add...*, replace *.sdf by *.mol and press *Enter*. Select your previously exported .mol file and click *OK*. Also click *OK* in the *Import SD files* dialog. Your molecule should now be in the database. However, a final step is required to match LigandScout's features: Select *Compute > Pharmacophores...* and choose *PHCD* in the drop-down menu next to *Scheme*. Click on *OK* and your database is all set.

Now, click on *Search...* in the *Pharmacophore Query Editor*. In the *Open* dialog choose your newly created database (*.mdb) and click *OK*. Click on *Search* and if everything worked out, you will see a status message saying "1 molecule, 1 hit". Otherwise, at least one feature did not match and needs some work. The check box *Enable Partial Match* in the *Pharmacophore Query Editor* allows you to find out how many features did not match by iteratively decreasing the number of features which need to match.

8.2.3. Preparing a Pharmacophore for Virtual Screening with Phase

Creating a Phase 2.0 compatible Pharmacophore

LigandScout is designed for the retrieval of automatically created [advanced 3D pharmacophore models](#) to broaden the scope of a single model. However, not all in-silico screening applications support exactly the same features or define their features in exactly the same way.

If you are using the software-package Phase, it is recommended to use the *Create Simplified Pharmacophore (Phase)* routine. The process is started by pressing CTRL+SHIFT+F10 (Shift+Command+F10 on MAC) or using *Pharmacophore > Create Simplified Pharmacophore (Phase)* . In this case, LigandScout achieves compatibility by reducing multiple H-acceptor or donor features at the same ligand atom

to a single feature of the respective type, and by implementing the hydrophobic feature perception algorithm of Phase. Hydrogen bonding interactions are exported as point features to overcome the lack of freely choosable bonding directions in combination with projected points and Phase's mandatory placement of H-donor features at the position of hydrogen atoms.

You may also reduce pharmacophores for Phase by manually deleting features. In this case, it is recommended to first delete features interacting with water molecules and then to erase features that have unfavorable distances and angles. Try to preserve the best feature for every heavy atom if there are multiple features of the same type on one heavy atom. You may also use literature to sort out interactions.

Exporting Pharmacophores for Use in Phase

Open the [Save](#) dialog in the *File* menu. Choose *Pharmacophore as Phase Files (*.xyz *.xvol *.def *.dxyz *.mae)* in the file type drop-down menu, enter a file name and export the pharmacophore. It is highly recommended to also export the ligand in a format readable by Phase for later hypothesis verification. Herewith, work in LigandScout is finished.

Importing a Hypothesis into Phase

Run Maestro and import your hypothesis `.xyz` file using the *Hypotheses Table* dialog reachable from the *Applications/Phase* menu. Afterwards, you can see the pharmacophore and excluded volumes as colored balls in the Maestro main window.

Verifying the Hypothesis in Phase

The following verification procedure assumes that the ligand structure has been exported from LigandScout as `ligand.sdf` and the corresponding Phase hypothesis has been saved under the name `hypothesis.xyz`. Furthermore, the hypothesis and ligand files have to be accessible from the current working directory.

1. Create a Phase database containing only the ligand:
`$SCHRODINGER/utilities/phasedb_manage -new sdf -db `pwd`/ligand_db -sd ligand.sdf -confs true cp hypothesis.def ligand_db_feature.ini $SCHRODINGER/utilities/phasedb_confsites -db `pwd`/ligand_db -JOB create_ligand_db`
2. Try to match the hypothesis with the ligand molecule:
`$SCHRODINGER/utilities/phasedb_findmatches -setup hypo_search -db `pwd`/ligand_db -hypo `pwd`/hypothesis -mode find+fetch -deltaDist 2 -useExistingSites true -useDbKeys false $SCHRODINGER/phase_dbsearch hypo_search`

Successful execution of the above commands results in a search log-file called `hypo_search_dbsearch.log`. Status messages near the bottom of the file should indicate a successful overall search procedure and that 1 match to the hypothesis has been found in the database.

Chapter 9. LigandScout Settings: Preferences

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LigandScout supports a plenitude of customizable settings. The *Preferences* can be accessed from the *Edit* menu on Linux and Windows systems, and from the *LigandScout* menu on Mac OSX platforms, respectively. This panel is composed of eight major settings areas:

1. [Structure-Based Modeling Settings](#)
2. [Ligand-Based Modeling Settings](#)
3. [Chemistry Settings](#)
4. [Library Screening Settings](#)
5. [Alignment Settings](#)
6. [Rendering 3D and 2D Settings](#)
7. [Program and Network Settings](#)

Every entry of the *Preferences* dialog exhibits a *Default* button and *All Default* button. The *Default* button sets the current preference tab to their default settings (e.g. PDB interpretation tab). *All Defaults* sets the whole *Preferences* dialog to factory defaults.

9.1. Structure-Based Modeling Settings

9.1.1. PDB Interpretation

The *PDB Interpretation Settings* allows for adjusting ligand interpretation and customization of properties of chemical features ([distance ranges](#), [hydrogen bonding](#), [metal binding](#), [hydrophobicity](#) and [aromatic interactions](#)) used in the pharmacophore generation.

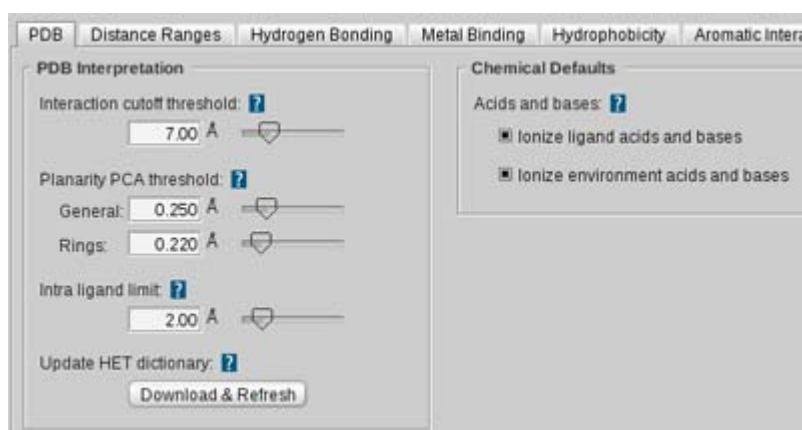


Figure 9.1. PDB interpretation

PDB Interpretation	Effect
Interaction cutoff threshold	Defines a sphere surrounding each non-hydrogen atom of the ligand. All non-hydrogen atoms in the environment which are within this sphere are considered as possible interaction partners during the pharmacophore generation process. (default: 7.00 Å)
Planarity PCA threshold	Defines the maximum out-of-plane deviation of parts of a molecule to be considered planar. <i>General</i> sets the threshold for acyclic molecule parts whereas <i>Rings</i> sets the threshold for cyclic molecule parts. Higher PCA threshold increases the out-of-plane tolerance resulting in an enhanced preference for sp^3 hybridized atoms. (default: General: 0.250 Å; Rings: 0.220 Å)
Intra ligand limit	Defines the maximum spacial distance between atoms of two molecules to be considered as a single ligand. Increasing this limit results in a preferred interpretation of two molecules to be seen as one ligand. (default: 2.00 Å)
Update HET dictionary	Updates the HET Dictionary from an online PDB resource. The HET dictionary is used to enhance the PDB interpretation in case the loaded data is incomplete (e.g. missing connect records, etc.)
Chemical Defaults	Effect
Acids and cases	If set true for the ligand or environment, the protonation states will be adjusted automatically after downloading or opening PDB structures. In fact, all single-bonded oxygens of acidic moieties will be deprotonated. For nitrogens which are double-bonded to two oxygens, one of the double-bonds will be replaced by a single bond,

Chemical Defaults	Effect
	the nitrogen's charge will be set to +1 and the oxygen will be deprotonated. (default: enabled)

9.1.2. Distance Ranges

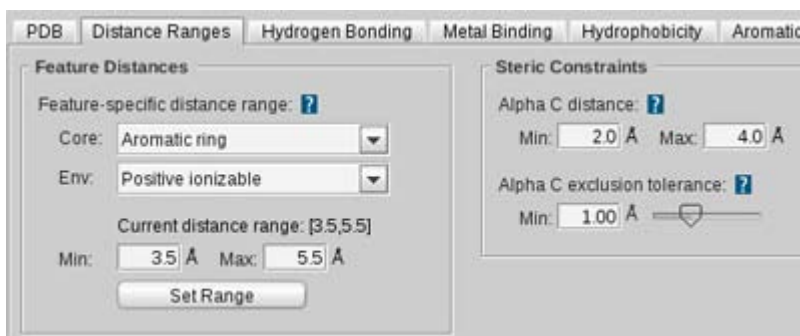


Figure 9.2. Distance ranges settings

Feature Distance	Effect
Feature-specific distance range	Defines the distance interval in which LigandScout considers hydrogen bonding by <i>Hydrogen Bond Donor</i> and <i>Hydrogen Bond Acceptor</i> features, hydrophobic interactions by <i>Hydrophobic</i> features, charge interactions by <i>Ionizable</i> features, metal binding interactions by <i>Magnesium</i> , <i>Zinc</i> , and <i>IronBinding Locations</i> , and aromatic interactions by <i>Aromatic Plane</i> features.
Core	Select the ligand-side feature for which the distance constraint should be set.
Env	Select the environment-side feature for which the distance constraint should be set. An interaction is only possible within the defined distance range. The environment is the part of the macro-molecule that surrounds the ligand.
Current distance range	Shows the currently set distance range for the selected core-environment feature pair.
Min	Adjust the minimum distance range. (in Å)
Max	Adjust the maximum distance range (in Å).
Set Range	Sets the newly adjusted distance range for the selected core-environment feature pair.
Steric Constraints	Effect
Alpha C distance	Set the minimum and maximum distance (such that an excluded volume on an alpha carbon can be generated. If the alpha carbon has interactions with ligand atoms closer than the maximum distance and no other feature is closer than the minimum distance, then an excluded volume will be created on an alpha carbon. (default: Min: 2.0 Å; Max: 4.0 Å)
Alpha C exclusion tolerance	Sets the tolerance value which an excluded volume on an alpha carbon will have. (default: 1.00 Å)

9.1.3. Hydrogen Bonding

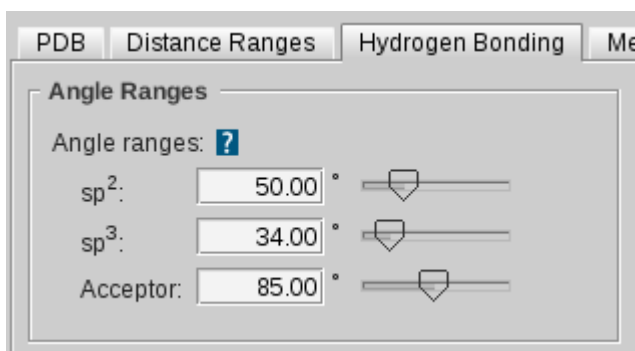


Figure 9.3. Chemical feature hydrogen bond settings

Angle ranges	Effect
sp ²	For an sp ² hydrogen bond donor atom. The angle between the vector (hbd atom, hydrogen) and vector (hydrogen, hba atom) must be below this threshold to be a valid hydrogen bond. (default: 50.00°)
sp ³	For an sp ³ hydrogen bond donor atom. The angle between the vector (hbd atom, hydrogen) and vector (hydrogen, hba atom) must be below this threshold to be a valid hydrogen bond. (default: 34.00°)
Acceptor	Calculate the weighted vector from the hydrogen bond acceptor atom to all of its neighbors. This mixed direction vector is inverted and checked against the vector (hydrogen bond acceptor atom, hydrogen bond donor atom). This angle must be below the specified threshold. (default: 85.00°)

9.1.4. Metal Binding

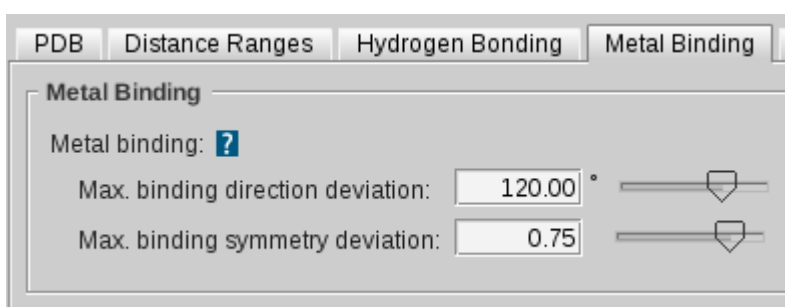


Figure 9.4. Chemical feature metal binding

Metal binding	Effect
Maximum binding direction deviation	Defines the maximum angle deviation from a ligating atoms idealized metal-binding axis. Lowering the value causes more restrictive handling of preferred binding direction deviations. (default: 120.00°)
Maximum binding symmetry deviation	Defines the maximum allowed relative binding length difference of a metal atom to two or more ligating atoms. This setting is only effective in case of chelating moieties like hydroxamates. The lower the value the more restrictive is the handling. (default: 0.75Å)

9.1.5. Hydrophobicity

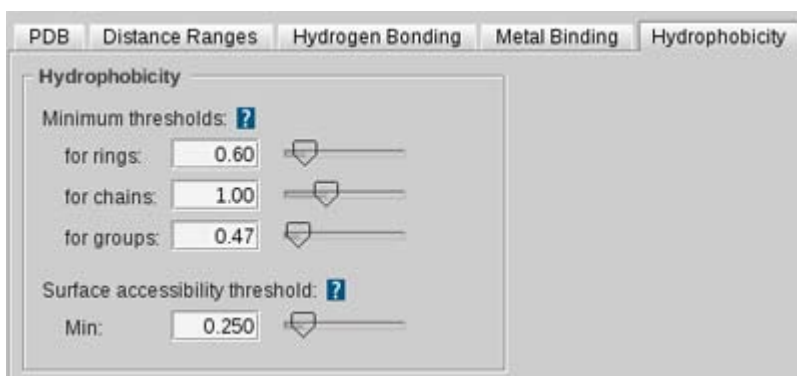


Figure 9.5. chemical feature hydrophobicity settings

Hydrophobicity	Effect
Minimum thresholds	Defines the minimum hydrophobic threshold of rings, chains and groups to be considered as a hydrophobic moiety. Increasing the minimum threshold causes more restrictive handling of hydrophobicity characteristics, resulting in pharmacophores containing less hydrophobic features.
Surface accessibility threshold	Defines the minimum sterical accessibility threshold for moieties to be considered as hydrophobic. The higher the threshold the more restrictive the feature identification will be.

9.1.6. Aromatic Interactions

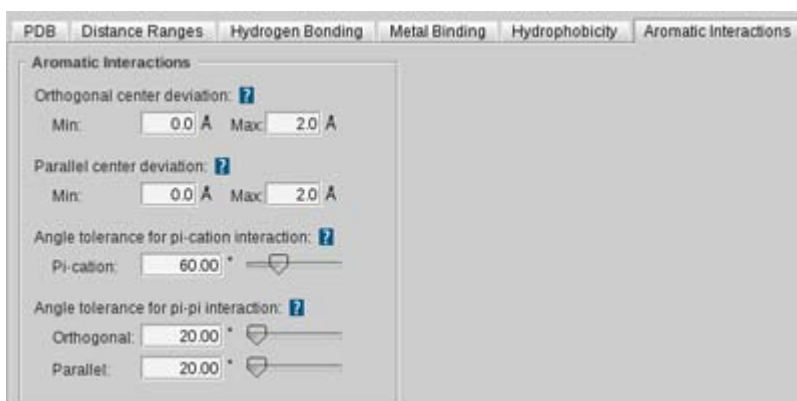


Figure 9.6. Chemical feature aromatic interaction settings

Aromatic Interactions	Effect
Orthogonal center deviation	The minimum and maximum distance constraint two orthogonal plane feature center points (one from the core and the other from the environment) must meet. (default: Min: 0.0 Å; Max: 2.0 Å)
Parallel center deviation	The minimum and maximum distance constraint two parallel plane feature center points (one from the core and the other from the environment) must meet. (default: Min: 0.0 Å; Max: 2.0 Å)

Aromatic Interactions	Effect
Angle tolerance for pi-cation interactions	The angle constraint that must be met for the angle between vector (plane center, ion position) and plane normal vector to be a valid plane ion interaction feature. (default: 60.00°)
Angle tolerance for pi-pi interactions	<i>Orthogonal</i> sets the maximum angle the two normal vectors for two pi-pi orthogonal plane features may enclose to be a valid interaction. <i>Parallel</i> sets the maximum angle the two normal vectors for two Pi-Pi parallel plane features may enclose to be a valid interaction. (default: orthogonal: 20.00°; parallel: 20.00°)

9.2. Ligand-Based Modeling Settings

9.2.1. Settings and Scoring

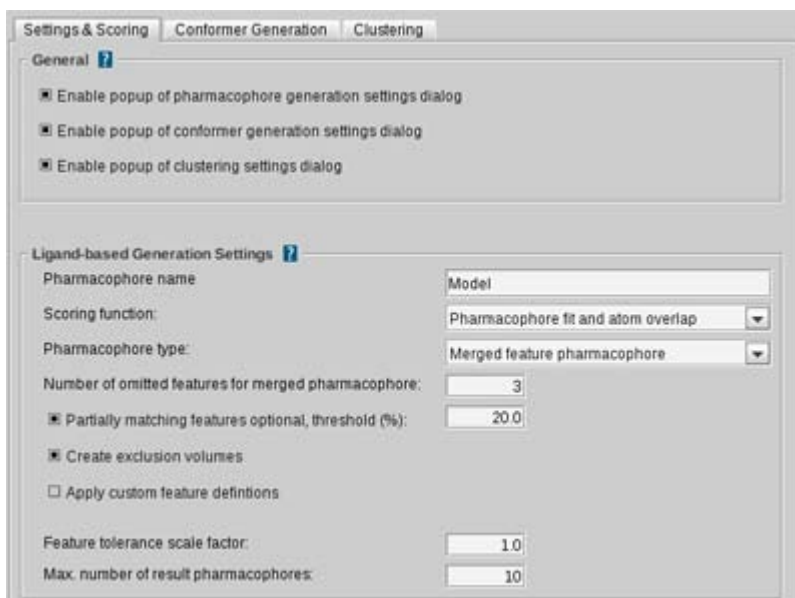


Figure 9.7. Settings and scoring

General	Effect
Enable popup	Enables/Disables the settings dialog popup for pharmacophore generation, conformer generation and clustering. (default: enabled)
Ligand-Based Generation Settings	Effect
Pharmacophore name	Sets the default name (prefix) for the resulting pharmacophores. (default: Model)

Ligand-Based Generation Settings	Effect
Scoring function	During the ligand-based pharmacophore generation all intermediate alignment results are scored according to the selected scoring function. The <i>pharmacophore fit</i> scoring function only considers pharmacophoric features and the feature RMS deviation. The <i>relative pharmacophore fit</i> scores the number of matching pharmacophore features and the RMSD of the pharmacophore alignment normalized to [0..1]. If you want to consider steric properties, you can choose the <i>atom sphere overlap (fast shape)</i> or <i>Gaussian shape similarity</i> scoring functions. The first implements a fast atom sphere counting algorithm to provide an overlap score. The latter, uses Gaussian functions to approximate atom spheres. By doing so the overlap can be calculated analytically. (default: Pharmacophore fit and atom overlap)
Pharmacophore type	Two different types of pharmacophoric models can be derived due to different generation processes. The ligand-based pharmacophore generation is a pair-wise process. In each step one pharmacophore for two molecules is drained. This we can do by either taking all common features (Shared Feature Pharmacophore) or by taking all features (Merged Feature Pharmacophore). In the latter case each feature is scored and those are removed that do not match all input molecules (see number of omitted features for merged pharmacophore). The <i>shared feature pharmacophore</i> creates a pharmacophore that represents all common features of a ligand-set. If you want to take all features into account and assemble them into one pharmacophore, then the <i>merged feature pharmacophore</i> is your right choice. (default: Merged feature pharmacophore)
Number of omitted features for merged pharmacophore	This setting specifies the number of features that need not match all input molecules if <i>Merged feature pharmacophore</i> type is turned on. (default: 3)
Partially matching features optional, threshold (%)	If this threshold is exceeded, e.g. more than 20% of the molecules do not match a feature, the feature is declared as optional, otherwise it is handled like a normal feature. (default: 20)
Create exclusion volumes	Excluded Volumes are generated. (default: enabled)
Apply custom feature settings	Enables customized feature definitions. (default: disabled)
Feature tolerance scale factor	This value is multiplied to the pre-defined feature tolerances. Lowering this scale factor would end up in more restrictive pharmacophoric models. (default: 1.0)
Max. number of resulting pharmacophores	The maximum number of pharmacophores to be generated. (default: 10)

9.2.2. Conformer Generation

Customizing *Conformation Settings* allow you to tweak *OMEGA* settings.

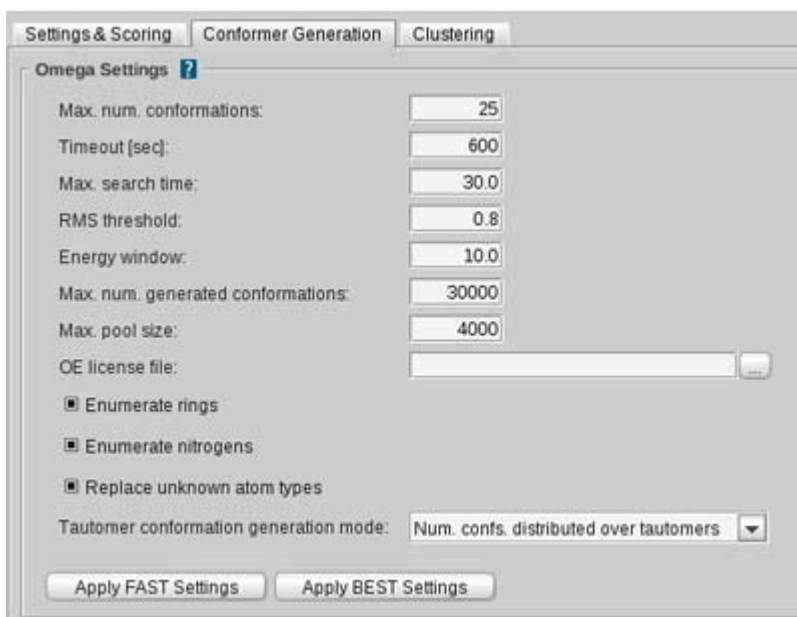


Figure 9.8. Conformer generation

Conformer Generation Settings	Effect
Max. num. conformations	Maximum number of conformations to output for each molecule. Raising this value samples the conformational space better. (default: 25)
Timeout [sec]	After this time, the conformer generation for a molecule is stopped (and is considered to have failed!). If this occurs in the ligand-based pharmacophore generation, the whole process automatically fails. (default: 600 sec)
Max. search time	The maximum time that is allowed for torsion search. (default: 30.0)
RMS threshold	RMS threshold used to determine duplicate conformations. (default: 0.8)
Energy window	Energy window used for conformer selection. (default: 10.0)
Max. num. generated conformations	Maximum number of conformations to generate during the whole process. (default: 30 000)
Max. pool size	Maximum size of intermediate pool of conformers. (default: 4000)
OE license file	The OMEGA license file to use (if left blank the built-in license is used).
Enumerate rings	If checked, ring conformers are enumerated. (default: enabled)
Enumerate nitrogens	If checked, pyramidal invertible nitrogen geometries are enumerated. (default: enabled)
Replace unknown atom types	If checked, atom types that are not supported by MMFF94 get replaced by closely supported analogues (e.g. Selenium by Sulfur). (default: enabled)
Tautomer conformation generation mode	Sets the mode to generate tautomer conformation (Number of conformations distributed over tautomers or per tautomer. (default: Num. confs. distributed over tautomers)
Apply FAST Settings	<i>Fast Settings</i> are the settings for <i>OMEGA</i> which will result in a fast generation of conformations but potentially of lower quality.

Conformer Generation Settings	Effect
Apply BEST Settings	<i>Best Quality Settings</i> are the settings which will result in longer processing time but higher quality resulting conformations.

9.2.3. Clustering

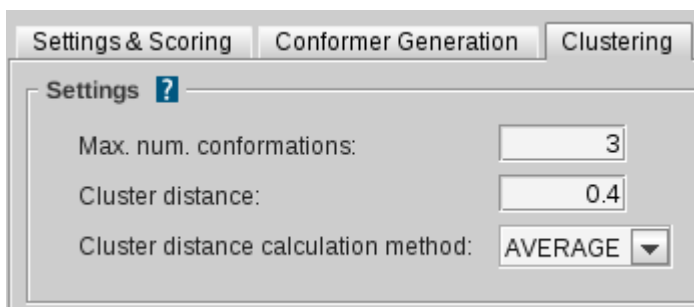


Figure 9.9. Clustering settings

Settings	Effect
Max. num. conformations	Defines the maximum number of conformations to take from the pool of available ligand conformations. (default: 3)
Cluster distance	Defines the cluster distance. Low distances results in many clusters. (default: 0.4)
Cluster distance calculation method	Defines the type of cluster distances to calculate (Maximum, Minimum or Average). (default: Average)

9.3. Chemistry Settings

9.3.1. Tautomerization

Tautomerization Settings allow for the customization of various parameters influencing ligand tautomer enumeration and filtering.

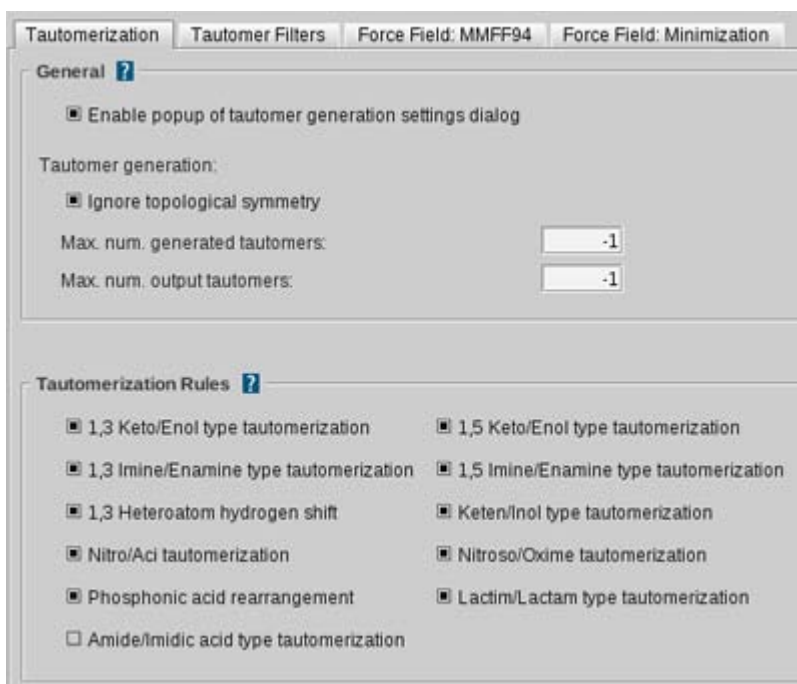


Figure 9.10. Tautomerization Settings

General	Effect
Enable popup of tautomer generation settings dialog	Enables the settings dialog of the tautomer generation. (default: enabled)
Ignore topological symmetry	If not checked, topologically identical ligand tautomers are discarded even if they are different in 3D structure. (default: enabled)
Max. num. generated tautomers	Specifies the maximum number of unfiltered tautomers to generate. A value of 0 or -1 means no limit. (default: -1)
Max. num. output tautomers	Specifies the maximum number of filtered output tautomers to generate. A value of 0 or -1 means no limit. (default: -1)
Tautomerization Rules	Effect
Tautomerization Rules	Allows you to enable/disable different kinds of common tautomeric rearrangements. If all rules are disabled, no tautomers are generated.

9.3.2. Tautomer Filters

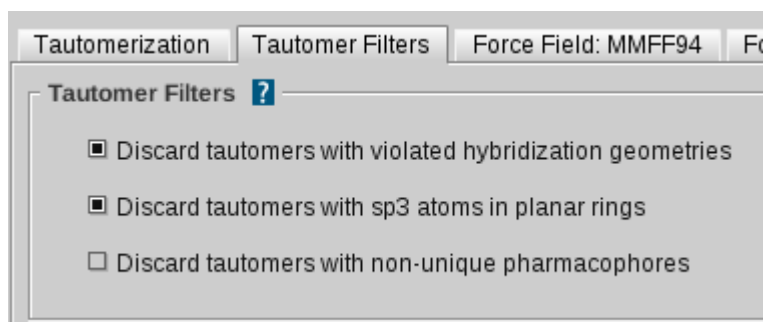


Figure 9.11. Tautomer filters

Tautomer Filters	Effect
Discard tautomers with violated hybridization geometries	If checked, tautomers with atom hybridization states that require a different spatial distribution of the neighbor atoms than the given one are discarded (e.g. sp^2 requires a trigonal planar arrangement of the neighbors but the given arrangement is trigonal pyramidal). The setting has only an effect if atom 3D coordinates are available. (default: enabled)
Discard tautomers with sp^3 atoms in planar rings	If checked, tautomeric forms of a molecule with sp^3 atoms in rings that have been perceived as aromatic in the start tautomer are discarded. (default: enabled)
Discard tautomers with non-unique pharmacophores	If checked, each generated tautomer with a molecule pharmacophore that is identical to a pharmacophore of a previous tautomeric form is discarded. (default: disabled)

9.3.3. Force Field: MMFF94

MMFF94 and Minimization Settings allow for the customization of various parameters influencing MMFF94 forcefield energy calculations and the conjugate gradients algorithm used for structure optimization.

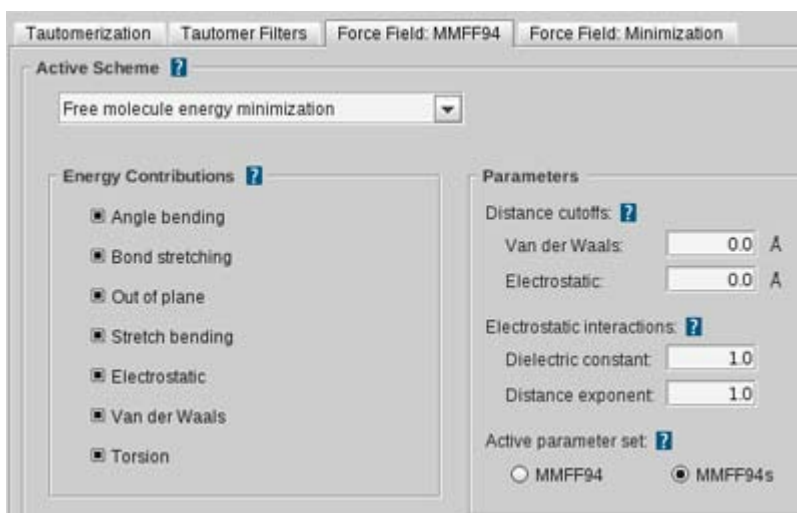


Figure 9.12. Force field: MMFF94

Active Scheme	Effect
Active Scheme	Specifies the settings scheme depending on different cases. <i>Binding pocket molecule energy minimization</i> : If the molecule lies in the active site. <i>Free molecule energy minimization</i> : the ligand without environment. <i>Library molecule energy calculation</i> : calculates energies of molecules in a library. (default: free molecule energy minimization)
Energy Contributions	Effect
Energy Contributions	Specifies which types of forcefield interactions are considered and contribute to the overall MMFF94 energy.
Angle bending	If checked, angle bending interactions will contribute to the total energy. Otherwise angle bending is ignored. (default: enabled)
Bond stretching	If checked, bond stretching interactions will contribute to the total energy. Otherwise bond stretching is ignored. (default: enabled)
Out of plane	If checked, out of plane bending interactions will contribute to the total energy. Otherwise out of plane bending is ignored. (default: enabled)
Stretch bending	If checked, stretch bend interactions will contribute to the total energy. Otherwise stretch bend interactions are ignored. (default: enabled)
Electrostatic	If checked, electrostatic interactions will contribute to the total energy. Otherwise Coulomb interactions are ignored. (default: enabled)
Van der Waals	If checked, van der Waals interactions will contribute to the total energy. Otherwise van der Waals interactions are ignored. (default: enabled)
Torsion	If checked, torsion interactions will contribute to the total energy. Otherwise torsion interactions are ignored. (default: enabled)
Parameters	Effect
Distance cutoffs	Allows the specification of distance cutoffs for van der Waals and electrostatic interactions. A value of zero disables the interaction distance cutoff.

Parameters	Effect
Van der Waals	Defines the distance between two atoms up to which a van der Waals interaction is considered to be present. (default: 12.0 Å)
Electrostatic	Defines the distance between two atoms up to which an electrostatic interaction is considered to be present. (default: 0.0 Å)
Electrostatic interactions	Effect
Electrostatic interactions	Definition of the distance exponent and dielectric constant used for electrostatic interaction energy calculations.
Dielectric constant	Specifies the dielectric constant to use for the calculation of Coulomb interaction energy contributions. (default: 1.0)
Distance exponent	Specifies the exponent of the interatomic distance in Coulomb interaction terms. (default: 2.0)
Parameter Set	Effect
Active parameter set	Specifies the parameter set to be used for calculation of MMFF94 energies. (default: MMFF94s enabled)
MMFF94	If selected, the 'dynamic' parameter set will be used.
MMFF94s	If selected, the 'static' parameter set will be used.

9.3.4. Force Field: Minimization

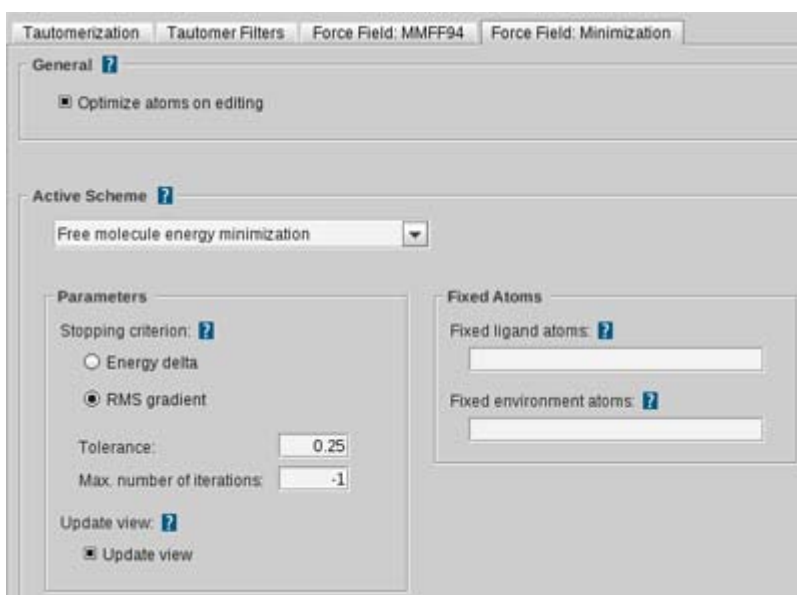


Figure 9.13. Force field: minimization

General	Effect
Optimize atoms on editing	If selected, the edited atoms and bonds (type change, deletion, addition) are optimized automatically. (default: enabled)

Active Scheme	Effect
Active Scheme	Specifies the settings scheme for energy minimization whether a ligand is located in a binding site or is solely available. (default: free molecule energy minimization)
Stopping Criteria	Effect
Stopping criterion	Selection of the optimization algorithm stopping criterion. (default: RMS gradient)
Energy delta	If selected, the tolerance value specifies a difference in energy between two optimization iterations. If the calculated energy difference is lower than the specified delta, the optimization is stopped. (default: disabled)
RMS gradient	If selected, the tolerance value specifies the root mean square of the energy gradient components. If the calculated RMS gradient is lower than the specified value, the optimization is stopped. (default: enabled)
Tolerance	Defines the value of the RMS gradient or energy delta (see above). (default: 0.25)
Max. number of iterations	Defines the maximum number of optimization iterations to perform. A value of -1 specifies that the optimization is performed until the rms gradient or energy delta is reached. (default: -1)
UpdateView	If checked, the 3D View will be updated while performing the structure optimization. (default: enabled)
Fixed Atoms	Effect
Fixed Atoms	Definition of SMARTS patterns for the specification of atoms whose position has to be kept fixed during a structure optimization run. In a complex SMARTS pattern (two or more connected atom-patterns matching a substructure) the atoms to keep fixed can be marked by curly braces, e.g. O={C}-O. Otherwise all matched atoms will be fixed.
Fixed ligand atoms	A whitespace separated list of SMARTS patterns describing the ligand atoms to fix.
Fixed environment atoms	A whitespace separated list of SMARTS patterns describing the environment atoms to fix. (default for binding pocket energy minimization: * {[#1]}-[!O,!H2])

9.4. Library Screening Settings

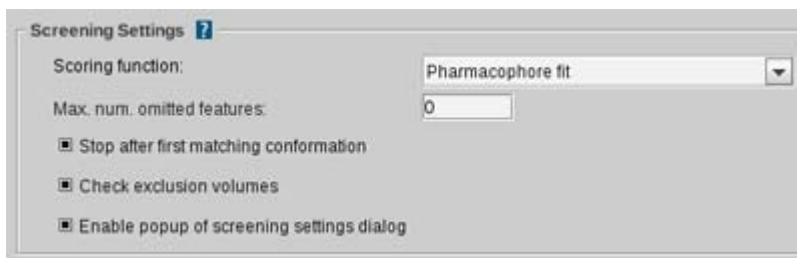


Figure 9.14. Library screening settings

Screening Settings	Effect
Scoring function	Choose the Scoring Function type that evaluates the alignments. (default: pharmacophore fit)
Max. num. omitted features	Defines the maximum number of features that can be omitted during the screening. If zero is specified, the whole pharmacophore must be matched to be a valid hit. (default: 0)
Stop after first matching conformation	If checked, the screening for a molecule stops when the first matched conformation is found. Note: this match is not the optimal one. (default: enabled)
Check exclusion volumes	Enables the consideration of exclusion volumes during the screening process. (default: enabled)
Enable popup of screening settings dialog	Enables the settings dialog of the screening process. (default: enabled)

9.5. Alignment Settings

The *Alignment Settings* allow customizations for the alignment of arbitrary combinations of molecules and/or pharmacophores. These settings are applied, depending on the active scheme.

9.5.1. Alignment

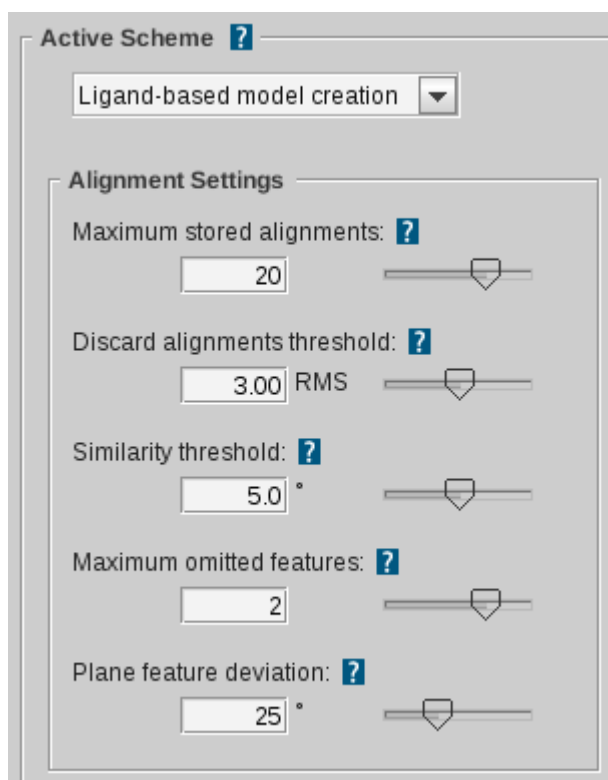


Figure 9.15. Alignment settings

Alignment	Effect
Active scheme	Specifies the settings scheme for alignments in the screening process, ligand-based model creation or Alignment Perspective. (default: virtual screening)
Maximum stored alignments	Defines the maximum number of stored alignments. In case there will be more alignments during the execution of the alignment algorithm, only the best will be kept and the others will be discarded. Note: The so called pharmacophore fit scoring function (considers number of features and RMS) is used. The scoring function can be customized only in the ligand-based settings to generate ligand-based pharmacophores. (default for Alignment Perspective and ligand-based model creation: 20; Screening: 1)
Discard alignments threshold	Defines the maximum allowed RMS (root mean squared) deviation of structures to be considered alignable. Alignments exceeding this limit are rejected. The RMS calculation is based on valid matched feature pairs of both structures. (default: 3.00 RMS)
Similarity threshold	For every generated alignment a rotation is stored. Depending on the deviation of the three unit vectors under this mapping an alignment will be discarded if it is too similar to a previous one. Certainly, it will be checked which alignment has the better RMS value and this one will be kept. (default: 5.0)
Maximum omitted features	Defines how many features can be omitted during alignment. Alignments will be generated by combinatorially excluding the specified number of features. This means that the more features are left out, the longer the generation process will take (NOTE: exponential growth). (default: 1)

Alignment	Effect
Plane feature deviation	Defines the maximum deviation of the normal vectors of two plane features to be included in an alignment. If the angle between the normal vectors exceeds the specified value, there will be no valid match between these two plane features. (default: 25°)

9.6. Rendering Settings

The *Renderer Settings* allow users to predefine default render styles for the 3D Viewer.

9.6.1. 3D General Settings

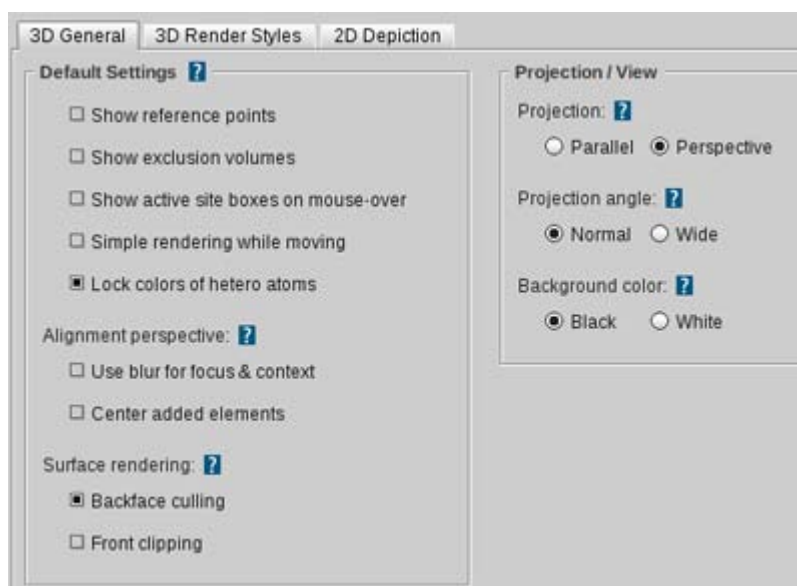


Figure 9.16. 3D general settings

Default Settings	Effect
Show reference points	Toggles on/off the default visibility of the reference points in the Alignment Perspective. If a pharmacophore or molecule is shown for the first time then the visibility of reference points is assigned according to this flag. (default: disabled)
Show exclusion volumes	Toggles on/off the default visibility of the exclusion volumes in all perspectives except the Structure-Based Modeling Perspective and Bookmark View. If a pharmacophore is shown for the first time then the visibility of exclusion volumes is assigned according to this flag. (default: disabled)

Default Settings	Effect
Show active site boxes on mouse-over	Enables/disables the Active Site boxes while moving the mouse over a ligand in the Macromolecule View. (default: disabled)
Simple rendering while moving	If turned on the render style is temporarily switched to line rendering while user interaction (i.e. rotating or translating the visible structure). This feature is especially designed for slower graphics hardware. (default: disabled)
Lock colors of hetero atoms	If enabled, the original colors of hetero atoms remain and cannot be changed. (default: enabled)
Alignment Perspective	Effect
Use blur for focus and context	The check box <i>use Blur</i> toggles between blurring and desaturated coloring of ligands and pharmacophores. (default: disabled)
center added elements	The check box <i>Center Elements</i> toggles centering of the ligand or of the pharmacophore while adding to the Alignment Panel. If enabled, the original coordinates are destroyed and the molecule or pharmacophore is centered in the origin of the model coordinate frame. This behavior can be undesirable if already aligned elements are added from the Alignment Perspective to another view. If you intend to add these elements back, e.g. to the binding pocket, the original coordinates should be retained and this feature should be turned off. (default: disabled)
Surface rendering	Effect
Backface culling	Enables/Disables the backface culling. If enabled all parts of a surface not facing the viewer will not be rendered. (default: disabled)
Front clipping	Enables/Disables the front clipping. If enabled then the surface area closest to the point of view is not rendered. (default: disabled)
General	Effect
Projection	Switch between parallel and perspective projection. (default: perspective)
Projection angle	Switch between normal and wide angle projection. The latter one enhances perspective distortion. (default: normal)
Background color	Switch between black and white background color scheme. (default: black)

9.6.2. 3D Renderer Style

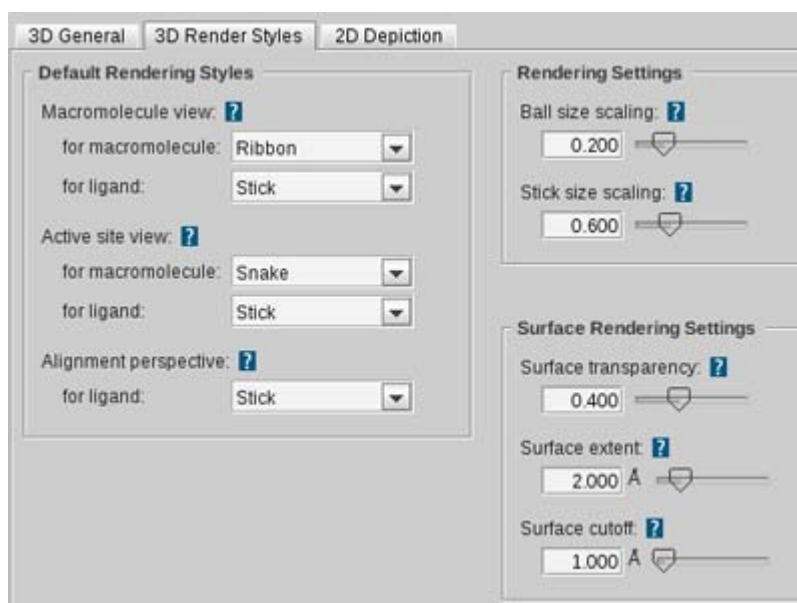


Figure 9.17. Renderer style

Default Rendering Styles	Effect
Macromolecule View	Defines the default render styles for macromolecule and ligand in the Macromolecule View . (default: macromolecule: ribbon; ligand: stick)
Active Site View	Defines the default render styles for macromolecule and ligand in the Active Site View . (default: macromolecule: snake; ligand: stick)
Alignment Perspective	Defines the default render styles for ligands and pharmacophores in the Alignment Perspective . (default: stick)
Rendering Settings	Effect
Ball size scaling	Defines a scaling factor which is applied to the ball size representing atoms. (default: 0.200)
Stick size scaling	Defines a scaling factor which is applied to the stick size representing bonds. Depending on this factor the "aromatic ring" thickness is adjusted too. (default: 0.600)
Surface Rendering Settings	Effect
Surface transparency	If switched on, you can see-through the generated surface. (default: 0.400)
Surface extent	This function is only referred to the receptor binding pocket option. The radius of every core atom is calculated whereas the nearest environment atom according to a core atom is taken into account. From that point on, the extent value determines the region (band with) of environment atoms that are included in the calculation of the surface. (default: 2.000 Å)

Surface Rendering Settings	Effect
Surface cutoff	Defines the distance of every core atom to neighboring environment atoms where the surface is cropped. A value of zero means that the surface is cut by the first environment atom. Rising up this value determines the distance from that point. The surface cutoff is only used for receptor binding pockets. (default: 1.000 Å)

9.6.3. 2D Depiction

The *2D Depiction Settings* allow one to adjust parameters for the 2D depiction of ligand structures.

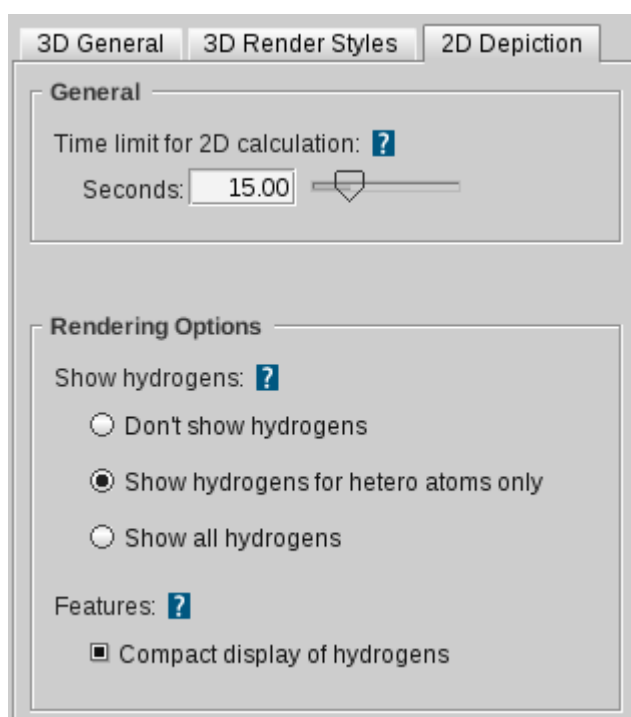


Figure 9.18. 2D depiction

General	Effect
Time limit for 2D calculation	Defines the maximum calculation time for 2D structures. 2D structure calculation will stop at this limit even if no structure could be retrieved yet. (default: 15.00 sec)
Rendering Options	Effect
Show hydrogens	Specifies which hydrogens should be shown in the 2D depiction. This can be either no hydrogens at all, hydrogens at hetero atoms only, or all hydrogens. (default: show hydrogens for hetero atoms only)
Features	Toggles compact display of hydrogens. If selected the hydrogens to be shown will be drawn as a label. Otherwise, hydrogens will be drawn with a bond to their neighbor atoms. (default: enabled)

9.7. Program and Network Settings

The *Program and Network Settings* allow to customize the direct web download of PDB structures.

9.7.1. Main Settings

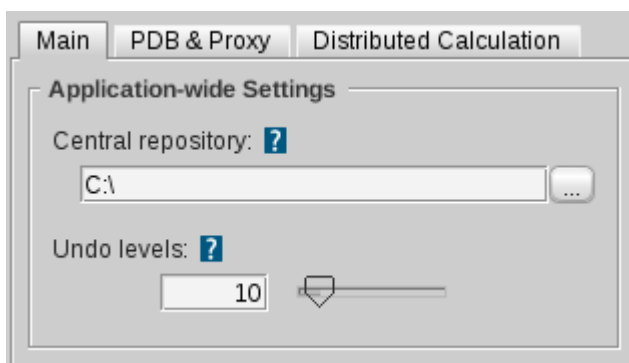


Figure 9.19. Main settings

Application-wide Settings	Effect
Central repository location	Sets LigandScout's Shared Repository path.
Undo levels	Sets the number of actions LigandScout can Undo/Redo. The higher this number, the more memory will be required to store information for Undo/Redo . (default: 10)

9.7.2. PDB and Proxy

The *PDB and Proxy Settings* allow to customize the direct web download of PDB structures.

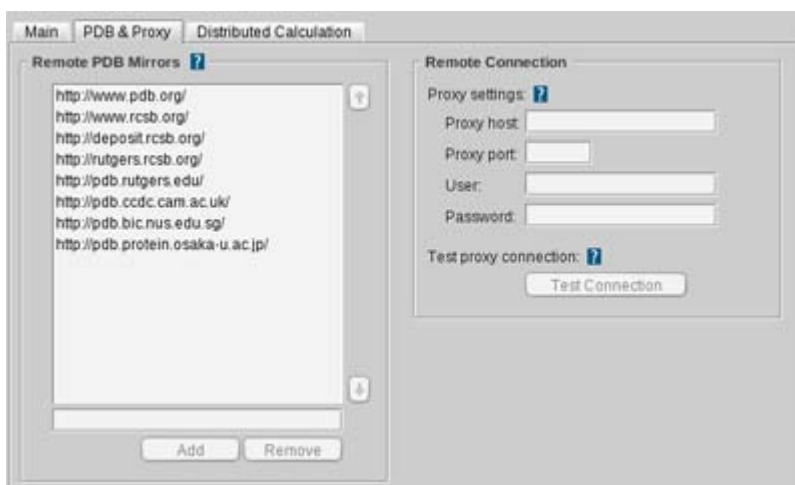


Figure 9.20. PDB and proxy

Remote PDB	Effect
Mirrors	Manage your favorite mirror servers for PDB download (add, remove, reorder). The list of mirror servers is seen as a prioritized list where the top most server is assigned the highest priority and subsequently the bottom most server is assigned the lowest priority. LigandScout will try to contact the servers and download the desired files from them according to the servers' assigned priorities.
Remote Connection	Effect
Proxy settings	Allows for web access using a proxy server (which might be familiar to you from your web browser settings). Please ask your system administrator for access information via your proxy server.
Test proxy connection	This button allows you to test the connection to your proxy server.

Addresses of additional PDB servers can be obtained from <http://www.rcsb.org/pdb/> [http://www.rcsb.org/pdb/] or any other PDB mirror.

9.7.3. Distributed Calculation

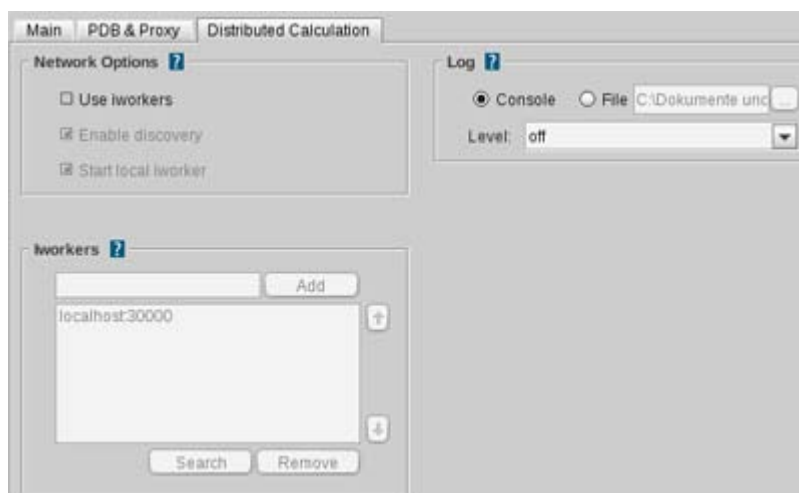


Figure 9.21. Distributed calculation settings

Network Options	Effect
Use iworkers	If enabled LigandScout uses network iworkers to speed up the screening process and the conformation generation. Please see Section 2.5, "Networking" . (default: disabled)
Enable discovery	If enabled, LigandScout tries to find iworkers in the network. (default: enabled)
Start local iworker	If enabled, the local iworker is automatically started. (default: enabled)
iworkers	Effect
iworkers	Add or remove iworkers by adding or removing their network addresses. A network address has the form HOSTNAME:PORT or

iworkers	Effect
	<p>IP_ADDRESS:PORT. HOSTNAME or IP_ADDRESS is the host name or ip-address of the iworker, and PORT is the TCP-port the iworker is listening to. (Remember that the system administrator has to open that port on the machine the iworker is running on!). Examples:</p> <ul style="list-style-type: none">• localhost:4283• foo.bar:8729• 192.168.0.34:5151• 135.27.1.119:1894
Log	Effect
Log	<p>If this option is used the logging of message traffic to and from this node will be shown in the console or redirected to the specified file. (default: console)</p>
Level	<p>The level defines how much information of the log will be provided. You can choose between <i>off</i>, <i>low</i>, <i>medium</i>, <i>high</i> and <i>highest</i>. Raising the level leads to a more detailed output. (default: off)</p>

Appendix A. Appendix

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A.1. LigandScout Menu Reference

LigandScout's menu bar consists of:

1. [File](#)
2. [Edit](#)
3. [Library](#)
4. [Ligand-Set](#)
5. [Molecule](#)
6. [Pharmacophore](#)
7. [Align & Merge](#)
8. [Render Control](#)
9. [Surface](#)
10. [Window](#)
11. [Help](#)

A.1.1. File

Open

Displays the [Open File](#) dialog (CTRL+O). Various file formats presenting chemical structure information (e.g.: *.pdb, *.sdf, ...) can be imported to LigandScout. If you open new data, all current data is closed before loading the new one.

Insert

This command allows the [injection of additional molecules](#) into your shown data (binding site and/or pharmacophore) in the *Structure-Based Modeling Perspective* (CTRL+I).

Save to Repository

[Save to Repository](#) (CTRL+S) stores active sites and macromolecule information in your Local Repository.

Save as File

Save as File (CTRL+SHIFT+S) enables you to [save/export](#) your macromolecule and ligand modifications as well as your pharmacophore models. Depending on the file format, check boxes in the [Save File dialog](#) let you choose advanced export settings and the items (ligand, environment, pharmacophore) you want to save/export. The export settings are sensitive according to the current data (e.g.: if no pharmacophore is present then related settings are disabled).

Close

Closes the shown data in the current view (CTRL+W). In the *Structure-Based Modeling Perspective* LigandScout gives feedback and asks for confirmation, in case there is modified or unsaved data.

Sessions: Load Session from File

Loads the previously exported SES file which contains data of the Local Repository, Alignment Perspective, Ligand-Based Modeling Perspective and Bookmark View.

Sessions: Save Session as File

Stores the current data of the Local Repository, Alignment Perspective, Ligand-Based Modeling Perspective and Bookmark View in a single session file (SES file).

Sessions: Store current Session

Stores the current data of the Local Repository, Alignment Perspective, Ligand-Based Modeling Perspective and Bookmark View in a single session file. Selecting your named session reloads all the data that was previously stored.

Sessions: Remove Session(s)

Deletes one of the available named session files from disk (irrevocable operation).

Repository: Delete from Local Repository

Deletes the current PDB complex from your Local Repository (CTRL+ALT+SHIFT+D). LigandScout will reload the original data from the PDB server if requested.

Repository: Import Repository

[Import Repository](#) (CTRL+ALT+SHIFT+I) allows you to import a zipped PMZ database (*.zip) either locally or to your default LigandScout data search path. The use of a PDB data repository drastically accelerates the ligand-macromolecule complex initialization, since LigandScout uses the structural information stored in the repository instead of downloading the file from the PDB server and interpreting it.

Repository: Export Repository

[Export Repository](#) (CTRL+ALT+SHIFT+E) allows you to export a zipped PMZ database (*.zip) either from the Local Repository or from your Shared Repository.

Repository: Clear Local Repository

[Clear Local Repository](#) (CTRL+ALT+SHIFT+C) erases the local PDB data storage.

Alignment: Import

Imports a previously exported set of alignments (CTRL+ALT+V). Using this functionality will directly add all stored molecules and pharmacophores to the [Alignment Perspective](#). In case the *Alignment Perspective* already contains elements, a dialog will pop up and ask if the old elements should be deleted before adding the new ones.

Alignment: Export

Export (CTRL+ALT+B) will export all molecules and pharmacophores which are currently listed in the [Alignment Perspective](#).

Print Preview

Print Preview (CTRL+ALT+P) shows the report that is printed with the *Print* command. Depending on the current view, *Print Preview* will show either a 3D image of the macromolecule if the application is in the Macromolecule View or it will show a report that will be printed with the *Print* command if the application is in the *Active Site View*.

In the Macromolecule View the result will look like:

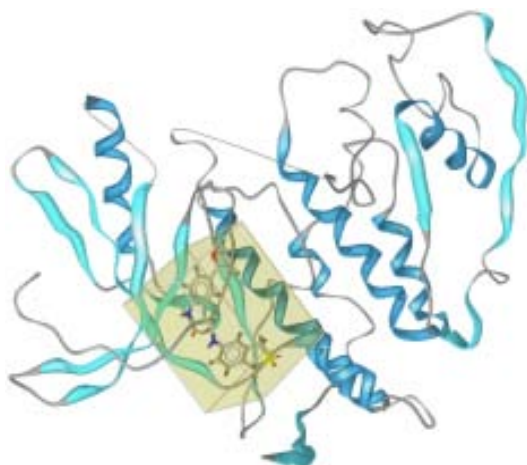


Figure A.1. Print preview of macromolecule

In the Active Site View the report which will show up contains a 3D image of the active site, a 2D depiction of the ligand as well as PDB complex meta data consisting of:

- PDB ligand key
- PDB complex ID
- Experimental type and resolution
- PDB classification
- Ligand name
- Deposition date
- Molecular formula

Here is an example of this report:

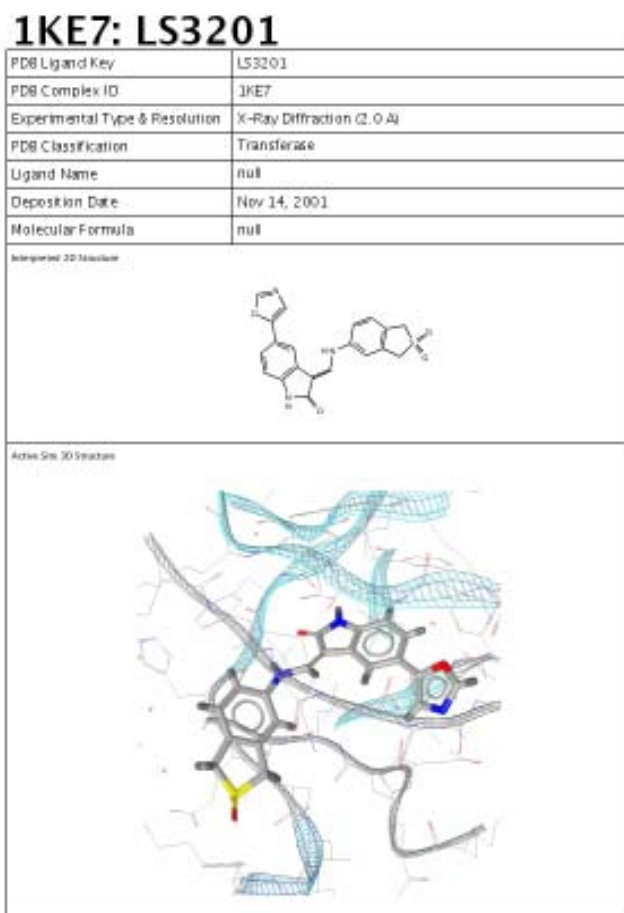


Figure A.2. Print preview of active site

Print

Print (CTRL+P) enables you to print out the current complex as a report containing a 3D image of the active site, a 2D depiction of the ligand as well as PDB complex meta data consisting of: PDB ligand name, complex identifier, PDB classification, etc. LigandScout uses white background for printing.

If you want to export the 2D and 3D View of your shown elements as images, for instance molecule alignments or single pharmacophore, select the *Save* command in the *File* menu. LigandScout supports a various list of image file types such as PNG, POV etc. For more information please see [Section 2.1.2, "Exporting Files"](#)

Restore Factory Defaults

Restore Factory Defaults (CTRL+SHIFT+F) will delete your Local Repository, alignment and bookmark lists, dictionaries and preference settings. In fact it will delete everything which is contained in the folder *.inteligand-data* (the application's working directory).

Recent Files

LigandScout journalizes the eight most recent files for fast and simple reaccess (CTRL+SHIFT+1...8).

Quit

Quits LigandScout application (CTRL+Q).

A.1.2. Edit

Undo

Undo (CTRL+Z) allows you to go back in history, reversing displays, selections and commands.

Redo

Redo (CTRL+Y) is the counterpart of the *Undo* command and executes your previously reversed action.

Select

Select: All (CTRL+A) picks all items (ligand, environment and pharmacophore) at once. The items can be individually selected by choosing “Ligand”, “Environment” or “Pharmacophore”.

Clear Selection

Clear Selection cancels all selections.

Invert Selection

Invert Selection inverts the current selection, i. e. this command selects all elements without the currently selected elements.

Delete Selected Elements

Delete Selected Elements (DEL) will delete all elements contained in the current selection.

Preferences [Linux/Windows]

The [Preferences](#) (CTRL+SHIFT+P) allow you to customize LigandScout settings. This item can be accessed via the *LigandScout* menu on Mac OSX platforms.

A.1.3. Library

Calculate Score to Shown Pharmacophore

Calculates the score between the shown pharmacophore and the ligands of the library. The pharmacophore score gives information about the fit between a pharmacophore and a molecule. This score takes into account the number of matched features times 10 and the RMSD (Root Mean Square Deviation).

Calculate Score to Shown Pharmacophore (Without Exclusion Volumes)

Calculate Score to Shown Pharmacophore (Without Exclusion Volumes) calculates the score between the shown pharmacophore and the ligands of the library. The exclusion volumes are not considered in score calculation.

Align and Calculate Score to Shown Pharmacophore

Align and Calculate Score to Shown Pharmacophore first aligns the molecules of the library with the shown pharmacophore. Then the score is calculated. The exclusion volumes are included in this score calculation. If a ligand extends into any of the exclusion volumes the score is set to -1.

Align and Calculate Score to Shown Pharmacophore (Without Exclusion Volumes)

Align and Calculate Score to Shown Pharmacophore (Without Exclusion Volumes) aligns first the molecules of the library with the shown pharmacophore. Then the score is calculated. The exclusion volumes are not included in the scoring process.

Calculate Standard Properties

Calculate Standard Properties calculates the standard properties of a molecule library. This includes the calculation of Rel. TPSA, TPSA [P.Ertl], number of rotatable bonds, acceptors, rings, cLogP, MolWt, heavy atoms and donors.

Calculate Gaussian Shape Similarity Score

Calculate Gaussian Shape Similarity Score uses Gaussian functions for analytically overlap calculations.

Calculate MMFF94 Strain Energy

Calculate MMFF94 Strain Energy calculates the MMFF94 strain energy using the [settings scheme](#) "Library Molecule Energy Calculation".

Calculate MMFF94 Binding Energy

Calculate MMFF94 Binding Energy calculates the MMFF94 binding energy of the ligand to the environment.

Clear User Properties

Clear User Properties removes all user calculated properties of the library table.

Clear File Properties

Clear File Properties prevents the display of all library properties that are contained in a loaded file.

Close Library

Close Library closes the *Library View*.

A.1.4. Ligand-Set

Add Molecules

Add Molecules adds additional molecules to your Training-Set, Test-Set or set of Ignored Ligands.

Flag Selected Molecules

Flag Selected Molecules marks selected molecules as Training-Set, Test-Set or set of Ignored Ligands. You can also decide, which molecules are active or inactive.

Remove Selected Molecules

Remove Selected Molecules deletes selected molecules from the Ligand-Set.

Remove non-Espresso Properties

Remove non-Espresso Properties deletes external properties of the Ligand-Set, that are not generated by Espresso.

Remove Pharmacophore Bias

Remove Pharmacophore Bias deletes the user-defined pharmacophore that influences the ligand-based pharmacophore generation.

(Re)Create Conformational Models

(Re)Create Conformational Models creates conformations to your Ligand-Set. If conformations already exist, they are recreated.

Cluster Ligand-Set

Cluster Ligand-Set clusters the Ligand-Set by pharmacophore based ligand similarity.

Create Pharmacophore

Create Pharmacophore automatically creates the pharmacophore, that is shared in the Training-Set and matches it with the Test-Set. Conformations are automatically generated for the molecules if not available.

Bias

Influences the Ligand-based pharmacophore generation according to pairwise alignment of pharmacophores in two ways. It can be influenced by a user-defined pharmacophore or customized feature definition.

A.1.5. Molecule

Re-Interpret Ligand

Re-Interpret Ligand (CTRL+R) causes LigandScout to re-interpret all bonds of the ligand shown in the current [Active Site View](#). This also triggers recalculation of properties like stereo chemistry, hydrogen positions, and aromaticity.

Move Selection to Ligand Side

Move Selection to Ligand Side (CTRL+SHIFT+C) adds the selected environment elements to the ligand side. LigandScout considers these elements as part of the ligand in pharmacophore generation. It is also possible in the [Macromolecule View](#) to select some amino acids or nucleic acids and move them to the core yielding new ligands instead of the acids bound to the macromolecule.

Move Selection to Macromolecule Side

Move Selection to Macromolecule Side (CTRL+SHIFT+E) adds the selected ligand elements to the macromolecule side. LigandScout considers these elements as part of the environment in pharmacophore generation. This entry will only be active in [Active Site View](#).

Create New Bond

Create New Bond (CTRL+SHIFT+B) adds a new bond between two selected atoms if it is *reasonable*. Essentially this means that two atoms can be bound together if their distance is not too short and not too long. This distance should be around the two atoms "normal" bonding distance.

Increase Charge of the Selected Elements

Increase Charge of the Selected Elements (+) adds a positive charge to the currently selected atoms.

Decrease Charge of the Selected Elements

Decrease Charge of the Selected Elements (-) adds a negative charge to the currently selected atoms.

Ionize Acids and Bases

Deprotonate all single-bonded oxygens of all acid-like substructures (CTRL+SHIFT+J). For nitrogens which are double-bonded to two oxygens one of the double-bonds will be replaced by a single bond, the nitrogen's charge will be set to +1 and the oxygen will be deprotonated. Basic amines, amidines, and guanidines are protonated.

Neutralize Acids and Bases

For all single-bonded deprotonated oxygens of acid-like substructures and all nitrogens which have a double bond to an oxygen and a single bond to a deprotonated oxygen, the deprotonated oxygens will be set back to a neutral state (protonated). Basic amines, amidines, and guanidines are neutralized (CTRL+SHIFT+K).

Move All Water Molecules and Metals to Ligand Side

Move All Water Molecules and Metals to Ligand Side (CTRL+SHIFT+L) relocates all metals and water molecules to the ligand side.

Move All Water Molecules and Metals to Macromolecule Side

Move All Water Molecules and Metals to Macromolecule Side (CTRL+SHIFT+M) relocates all metals and water molecules to the macromolecule side.

Minimize MMFF94 Energy

Minimize MMFF94 Energy (CTRL+ALT+O) minimizes the MMFF94 energy of the ligand. For an unbound ligand the MMFF94 and optimization settings scheme “Free Molecule Energy Minimization” applies. For a ligand within a binding pocket the settings scheme “Binding Pocket Molecule Energy Minimization” is used.

Generate Tautomers

Generate Tautomers (CTRL+ALT+G) performs a tautomer enumeration of the shown ligand.

Show Tautomers As Library

Show Tautomers As Library shows the tautomers that are associated with the ligand in *Library View*.

A.1.6. Pharmacophore

Create new Feature

Create new Feature can create three geometric types of features: vector (H-Bond Acceptor, H-Bond Donor, Iron Binding Location, Magnesium Binding Location, Zinc Binding Location), point (Hydrophobic, Negative Ionizable, Positive Ionizable, Exclusion Volume) and plane feature (Aromatic Ring). To create a vector feature between the ligand and the environment, select an atom on ligand-side and

on environment-side. Then, you can choose your appropriate feature. To create a feature only on the ligand-side, select at least one atom of the ligand. To create an aromatic ring feature, at least three atoms are needed to be selected. If you choose more than one atom (ligand-side only), the new feature is created on the average position of the selected atoms.

Change Feature Type

Change Feature Type alters the type of a feature. Select a feature and change its type. A submenu shows which types can be chosen.

Create Pharmacophore

Create Pharmacophore (CTRL+F9) activates LigandScout's automatic pharmacophore generation algorithm. Both ligand and environment characteristics are analyzed for pharmacophore generation.

Create Simplified Pharmacophore (Catalyst)

Create Simplified Pharmacophore (Catalyst) (SHIFT+F9) reduces [advanced 3D pharmacophore models](#) for [virtual screening with Catalyst](#). In case of multiple features on single atoms, LigandScout automatically obtains the best feature for Catalyst.

Create Simplified Pharmacophore (MOE)

Create Simplified Pharmacophore (MOE) (CTRL+SHIFT+F9) reduces [advanced 3D pharmacophore models](#) for [virtual screening with MOE](#). LigandScout will prepare the pharmacophore in a way which works best for MOE.

Create Simplified Pharmacophore (Phase)

Create Simplified Pharmacophore (Phase) (CTRL+SHIFT+F10) reduces [advanced 3D pharmacophore models](#) for [virtual screening with Phase](#). LigandScout will prepare the pharmacophore in a way which works best for Phase.

Clear Pharmacophore

Clear Pharmacophore (CTRL+ALT+ SHIFT+P) erases all pharmacophore features.

Create Exclusion Volume Coat

Create Exclusion Volumes Coat places [individually adjusted exclusion spheres](#) on the ligand surroundings occupied by protein residues. This feature allows one to simulate the binding site shape in order to significantly increase enrichment during virtual screening.

Create Exclusion Volumes on Selected Atoms

Create Exclusion Volumes on Selected Atoms places exclusion volumes on selected atoms.

Increase Selected Feature Tolerance by 0.15 Å

Increase Selected Feature Tolerance by 0.15 Å (CTRL+SHIFT+NumPad+) expands the [tolerance spheres](#) of pharmacophoric features by 0.15 Å in order to decrease the restrictivity of the pharmacophore model.

Decrease Selected Feature Tolerance by 0.15 Å

Decrease Selected Feature Tolerance by 0.15 Å (CTRL+SHIFT+NumPad-) narrows the [tolerance spheres](#) of pharmacophoric features by 0.15 Å in order to increase the restrictivity of the pharmacophore model.

Increase Selected Feature Weight by 0.1

Increase Selected Feature Weight by 0.1 rises the weight of a selected feature. The feature weight has an impact on the pair-wise alignment of pharmacophores. (maximum: 1)

Decrease Selected Feature Weight by 0.1

Decrease Selected Feature Weight by 0.1 lowers the weight of a selected feature. The feature weight has an impact on the pair-wise alignment of pharmacophores. (minimum: 0.1)

Interpolate Features

Interpolates selected features in the [Alignment Perspective](#) (CTRL+E). The features *must be of the same type* and *must belong to the same pharmacophore* to yield a new chemical feature replacing the selected old ones. Features will only be interpolated if their maximum pairwise distance will be lower than five times the average tolerance of the selected features.

Mark Features as

Mark Features as Optional flags the selected features which are not mandatory to find a valid alignment (similar to the max. omitted feature action). During the screening process, a molecule is still a valid hit when these marked features are not matched. *Unmark Features as Optional* reverses the flag of the selected features. To switch off features, select *Mark Features as Disabled*. These features are not taken into account in the alignment process. To switch disabled features on, select *Mark Features as Enabled*.

Screen Pharmacophore Against External Library

Use this functionality if you want to screen the current pharmacophore model against a molecule library which is not loaded in LigandScout. The *Open* dialog appears where you can choose the library and customize the [settings for the screening procedure](#).

A.1.7. Align & Merge

Center Coordinates of All Structures

Centers all shown elements in *Alignment Perspective* (CTRL+ALT+C).

Set Reference

Sets the selected ligand or pharmacophore explicitly as reference for the alignment procedure in the [Alignment Perspective](#) (CTRL+ALT+R).

Clear All Elements

Clear All Elements (CTRL+ALT+X) deletes all molecules and pharmacophores which are currently listed in the [Alignment Perspective](#). An confirmation pop up reconfirm your decision.

Align Selected Elements

Aligns the selected elements to the reference element ([Align by features](#)) or with respect to the protein environment ([Align by reference points](#)) in the *Alignment Perspective*. [Align by features](#) allows one to align both ligands and pharmacophore models by an algorithm examining the best possible pharmacophoric feature overlay. If no reference element was specified one of the selected elements will be chosen randomly. [Align by reference points](#) is a useful tool to investigate protein-ligand complexes of structurally related targets. It allows one to align the ligands with respect to the surrounding protein residues. In this way the user is able to directly compare the absolute position of one or several ligands within the aligned binding site.

Generate Shared Feature Pharmacophore

Allows one to generate [shared feature pharmacophores](#) based on [feature alignment](#) or [reference points alignment](#).

Align & Merge Pharmacophores

Generates [merged pharmacophores](#) based on [feature alignment](#) or the [alignment of reference points](#) of the selected pharmacophores and the pharmacophores of the selected molecules in the [Alignment Perspective](#). Every single chemical feature will be added (with its original positions) to a newly created pharmacophore, the so-called [merged pharmacophore](#).

Combine Features in One Pharmacophore

[Joins all features](#) of all selected pharmacophore models in one pharmacophore (CTRL+ALT+M).

Interpolate All Features Within Tolerances

In the [Alignment Perspective](#) this action will automatically group features of the same type which are within their mutual tolerances (CTRL+ALT+T). Every group of features will be independently interpolated yielding new features. These new features will replace the original groups of features in the resulting pharmacophore. In other words, the resulting pharmacophore will not contain features with an overlap of their tolerances that is too large.

A.1.8. Render Control

Ligand Default Style

The *Ligand Default Style* menu provides access to a variety of rendering modes, like *Line*, *Ball and Stick*, *Stick* and *Spacefilling/CPK*. Hydrogens can be excluded from depiction or be reduced to stubs. *Show Templates* activates the depiction of default geometric templates depending on the hybridization-state of the currently selected atom. *Show Interactions* displays all possible interaction partners within the macromolecule of the currently selected ligand atom. *Show Ionic Interactions* displays ionic interactions between metals and non-metals. The *Pickable* check box sets the ligand selectable/unselectable.

Environment Default Style

The *Environment Default Style* submenu of the *Render Control* menu corresponds in large part to the respective ligand menu and lets you select different rendering modes and define hydrogen handling. *Show Templates* activates the depiction of default geometric templates depending on the hybridization-state of the currently selected atom. The *Pickable* check box sets the environment selectable/unselectable in [Active Site View](#).

Selection

Selection lets you customize the rendering of the current selection. LigandScout provides four different modes: *Line*, *Ball and Stick*, *Stick* and *Spacefilling/CPK*.

Protein Backbone Default Style

The *Protein Backbone Default Style* submenu lets you control the rendering of the protein backbone. LigandScout provides four different modes: *Lines*, *Splines*, *Snake*, and *Ribbon*.

Pharmacophore

The *Pharmacophore* submenu enables you to switch on/off pharmacophore and excluded volume rendering. The *Pickable* check box makes the pharmacophoric features selectable or unselectable in 3D.

Alignment Perspective Defaults

Use Blur (SDOF: Semantic Depth of Field) allows advanced blurring of objects. The effect is only visible if the [corresponding check box](#) of ligands/pharmacophores in the Alignment Panel is activated. SDOF may cause graphical issues or slow down the performance on low end computer systems and is therefore recommended only with suitable hardware equipment. *Show Reference Points* allows to visualize the position of the protein residues when using [alignments based on reference points](#). *Show Reference Point Names* places residue identifiers to this reference points. *Show Feature Tethers* visualizes the affiliation of corresponding pharmacophoric features with tethers. *Show Reference Point Tethers* visualizes the affiliation of corresponding protein reference points with tethers.

Behavior

The *Behavior* submenu gives you control on the main [3D View](#) defaults. *Pickable Bonds* toggles the possibility to select bonds. *Highlight Bond Cylinders in Selection* will only show an effect in the render styles *Ball and Stick* and *Line*. If this option is disabled only the atom will be highlighted in *Ball and Stick* render style and nothing will be highlighted in *Line* render style. However, if this option is enabled a selected atom will also highlight all “outgoing” bonds half-way for both render styles. *Simple Rendering While Moving* (CTRL+V) causes LigandScout to use less complex rendering algorithms while moving the macromolecule-ligand complex. *Show Tooltips* shows you important information, such as classification, geometry, and coordinates of your currently selected atom, bond, or pharmacophore feature. Enabled option causes LigandScout to view a small information popup next to the selected object.

Projection

LigandScout supports parallel and perspective projection. *Wide Angle View* enhances perspective distortion. The *Light Background* option (CTRL+L) switches the 3D depiction to white background. *Depth*

Cueing enhances depth recognition in the scene. *AntiAliasing* neutralizes stairway-effects at the edge of rendered objects.

Center Camera on Ligand

Center Camera on Ligand (CTRL+Page Up) recenters the camera position on the ligand.

Center Camera on Binding Site

Center Camera on Binding Site (CTRL+Page Down) recenters the camera position on the protein binding site.

Turn Camera for Optimized View

Turn Camera for Optimized View recenters the camera position. This feature allows to quickly reposition the protein-ligand complex with minimum overlap of the ligand atoms.

Add Angle Monitor

Allows one to measure the angle between three selected atoms. You can erase the angle monitor by selecting the angle value and pressing the [Delete](#) icon.

Add Distance Monitor

Allows one to measure the distance between two selected atoms. You can erase the distance monitor by selecting the distance value and pressing the [Delete](#) icon.

A.1.9. Surface

None

No Surface will be displayed.

Ligand Van der Waals Surface

Depending on the van der Waals distances of ligand atoms spheres will be created and combined to the van der Waals surface (CTRL+ALT+SHIFT+W).

Ligand Solvent Accessible Surface

This surface describes the boundary which separates the ligand from its potential interacting atoms (CTRL+ALT+SHIFT+S). This means, points on this surface are the closest locations, where interacting atoms can appear.

Ligand Molecular Surface

This surface is created by placing a sphere around each ligand atom (CTRL+ALT+SHIFT+M). Then a hypothetical probe is rolled over these spheres. The record of where this probe touched the spheres will result in the molecular surface.

Receptor Binding Pocket

This will display the surface of all the atoms in the environment which potentially interact with ligand atoms (CTRL+ALT+SHIFT+B). However, only those parts of the surface facing the ligand will be drawn.

Hide Back Side (Backface Culling)

This option will toggle the visibility of parts of the surface which will be seen from behind. This is especially useful for viewing a receptor surface, because this will allow you to cut away the front part of the enclosing view and show the ligand as illustrated below:

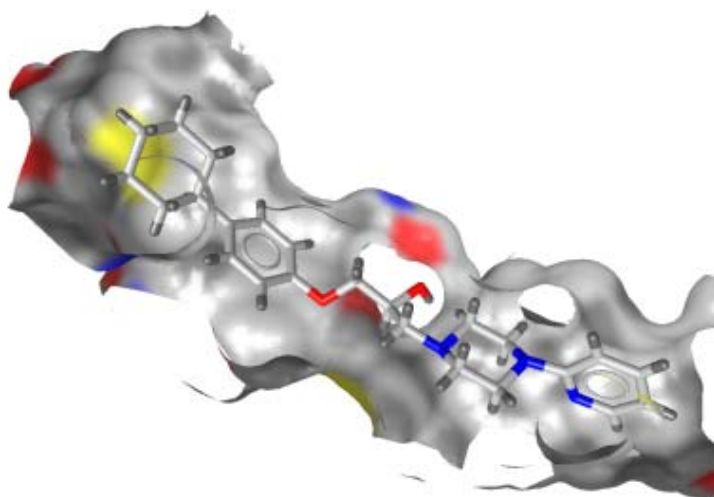


Figure A.3. Backface culling enabled (1HRV)

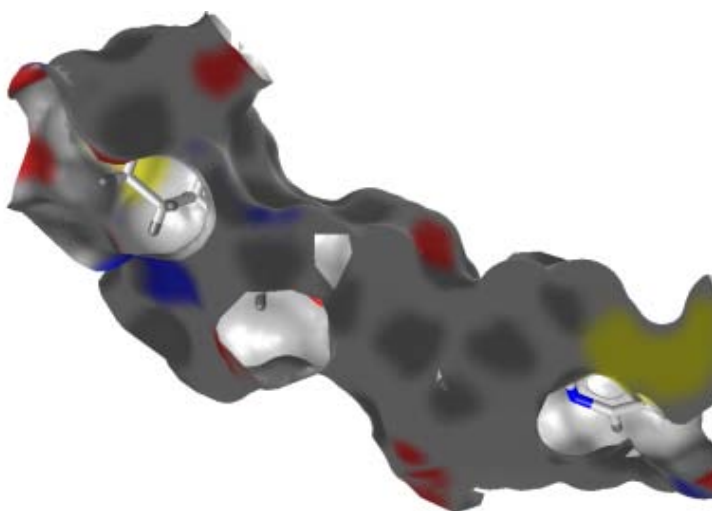


Figure A.4. Backface culling disabled (1HRV)

Transparency (Alpha Blending)

Activating this option will result in a surface rendered transparently such that for example also the interior of an enclosing surface will be visible.

Dynamically Update Surface

This option allows the fully automated recalculation of molecular surfaces upon molecule changes. The prerequisite for this option to have an effect is that a molecular surface type other than “None” is selected.

Front Clipping

Front clipping suppresses the visualization of parts of the surface that may avert a direct view of the ligand in the binding site. This feature is especially useful in the case of tight, elongated pockets.

Color Encoding

This menu item allows for coloring the surface. Available options are: *No Color-Coding (Gray)*, *By Atom Type*, *By Polarity*, *By Aggregated Lipophilicity/Hydrophobicity* and *By MMFF94 Partial Charge*.

- *No Color-Coding (Gray)* no specific coloring (defaults to gray).
- *By Atom Type* will use different colors for each atom type.
- *By Polarity* will color polar regions in red (hydrophilic areas).
- *By Aggregated Lipophilicity/Hydrophobicity* will color non-polar regions in yellow (hydrophobic areas).
- *By MMFF94 Partial Charge* will color by MMFF94 partial charge properties.

Render Style

This menu item allows to choose between different render styles like solid, wireframe or rendering points.

Recalculate & Update Surface

If “Dynamically Update Surface” is disabled, this menu item allows to recalculate molecular surfaces on request (CTRL+SHIFT+U). This may be needed if the core molecule was modified (e.g. changing of atom types, moving parts to core or to environment, ...).

A.1.10. Window

PDB/Alignment/Bookmarks

Depending on the perspective you can enable/disable the *PDB Control*, *Library/List*, *Alignment* and *Bookmark Panels*. The [PDB Control Panel](#) gives you information about the current PDB file and lets you select a ligand in the [Active Site View](#). The *Library/List Panel* shows molecules contained in a library. The [Alignment Panel](#) allows you to align ligands and pharmacophores. In the *Bookmarks Panel* you can save the current *Active Site View*.

2D Depiction

The *2D View Panel* shows the 2D structure and pharmacophore of the currently selected ligand (*Ligand 2D View*). Important information and statistics are shown as *Ligand Details View* in *Structure-Based Modeling Perspective* and *Alignment Details View* in *Alignment Perspective*. You can switch the *2D View Panel* on/off by using the keyboard shortcut CTRL+2.

Hierarchy View

The [Hierarchy View](#) (CTRL+T) gives a clearly arranged tree list of the currently visible elements (either the whole macromolecule or the active site, ligands and pharmacophores). You can also toggle visibility and colorize elements of the *3D View*.

Library Table

The [Library View](#) allows to examine an external compound library of small organic molecules. It is especially useful for the investigation of docking results in the [Structure-Based Modeling Perspective](#).

Ligand-Set Table

The [Ligand-Set Table](#) shows the ligands (Test-Set, Training-Set or Ignored Ligands) that are used for the ligand-based pharmacophore generation in the [Ligand-Based Modeling Perspective](#).

Screening Hits

The *Screening Hits* shows the hits of a screening run in the [Screening Perspective](#).

3D Widgets

Enables/Disables several types of widgets to exchange and navigate through your data. The [Copyboard Widget](#) transfers ligands, pharmacophores and active sites between perspectives. The [Zoom Out Widget](#) switches from the [Active Site View](#) to the [Macromolecule View](#). The [Alternatives Switcher](#) helps you to go through your collection of selected compounds in the Library View. The *Tautomer Switcher* and the *Conformer Switcher* guides you through the tautomers or conformers of a specific compound.

Show All Views and Tables

Show All Views and Tables (CTRL+SHIFT+A) enables all panels and control windows depending on the current view (*Ligand-Based Modeling Perspective*, *Structure-Based Modeling Perspective*, *Alignment Perspective*, *Bookmark View* or *Screening Perspective*).

Show 3D View Only

Show 3D View Only (CTRL+SHIFT+V) hides all other panels except *3D View*.

A.1.11. Help

LigandScout Help

LigandScout Help (F1) starts the built-in help system. The chapters describes the functions that are provided by LigandScout. You can also search specific terms in the *Help* dialog.

About this System

Displays information about this system's OpenGL implementation.

About LigandScout [Linux/Windows]











Displays information about LigandScout and the development team. The corresponding entry is located in the *LigandScout* menu on Mac OSX platforms.

License Information

Gives information about your LigandScout license.

A.2. LigandScout Icons Reference

A.2.1. Toolbar

	Undo		Change Atom Type
	Change Bond Type		Create New Bond
	Re-Interpret Core Molecule		Increase Charge
	Decrease Charge		Delete
	Center Ligand Molecule		Center Ligand and Environment
	Move to Environment		Move to Core
	Create Pharmacophore		Toggle Exclusion Volume Spheres Visibility
	Change Render Style of Selected Atoms		Toggle Hydrogen
	Change Macromolecule Render Style		Toggle Tooltips
	Add Angle Monitor		Add Distance Monitor

Please note that not all icons will be displayed in case of too small screen width. Depending on their importance the icons will be shown or not.

A.2.2. Hierarchy View



Collapse All



Toggle Visibility



Link with Selection



Change Color

A.2.3. Copyboard Widget



Copy current Ligand(s) to



Copy current binding site to



Copy current Pharmacophore to

A.2.4. Alignment Panel



Center All Visible Structures



Set Reference



Align Selected Elements



Generate Shared Feature Pharmacophore



Merge Pharmacophores and Interpolate Overlapping Features



Delete



Unselected



Selected



Context Representation



Focused Representation

A.2.5. Bookmarks Panel



Delete Bookmark



Bookmark

A.2.6. Library View

Align Molecules on Injection
into the Binding Site

Hide All Compounds

A.2.7. Library: Alternatives Switcher



Show Previous Compound



Show Next Compound



Show All Compounds

A.2.8. Ligand-Based Modeling Perspective Toolbar

Generate Conformations for
Ligand-Set

Clustering

Run Ligand-Based Pharma-
cophore Creation

Stop

A.2.9. Screening Toolbar



Load Database



Perform Screening



Plot ROC Curve



Delete Pharmacophore

A.2.10. Preferences Settings



Structure-Based



Ligand-Based



Chemistry



Screening



Alignment



Rendering



Program and Network

A.3. LigandScout Keyboard Shortcuts

Windows/Linux	Mac OSX	Function
CTRL+O	Command+O	open file
CTRL+I	Command+I	insert file
CTRL+S	Command+S	save to repository
CTRL+SHIFT+S	Command+SHIFT+S	save as file
CTRL+W	Command+W	close current data
CTRL+ALT+SHIFT+D	Option+SHIFT+Command+D	delete from Local Repository
CTRL+ALT+SHIFT+I	Option+SHIFT+Command+I	import repository
CTRL+ALT+SHIFT+E	Option+SHIFT+Command+E	export repository
CTRL+ALT+SHIFT+C	Option+SHIFT+Command+C	clear Local Repository
CTRL+ALT+V	Option+Command+V	import alignment
CTRL+ALT+B	Option+Command+B	export alignment
CTRL+ALT+P	Option+Command+P	print preview
CTRL+P	Command+P	print
CTRL+SHIFT+F	SHIFT+Command+F	restore factory defaults
CTRL+SHIFT+1...8	SHIFT+Command+1...8	open recent file
CTRL+Q	Command+Q	quit LigandScout
CTRL+Z	Command+Z	undo
CTRL+Y	Command+Y	redo
CTRL+A	Command+A	select all
DEL	Function+Backspace	delete selected elements

Windows/Linux	Mac OSX	Function
CTRL+SHIFT+P	Command+SHIFT+P	open preferences dialog
CTRL+R	Command+R	re-interpret ligand
CTRL+SHIFT+C	SHIFT+Command+C	move selection to ligand side
CTRL+SHIFT+E	SHIFT+Command+E	move selection to macromolecule side
CTRL+SHIFT+B	SHIFT+Command+B	create new bond
+	+	increase charge of selected elements
-	-	decrease charge of selected elements
CTRL+SHIFT+J	SHIFT+Command+J	ionize acids and bases
CTRL+SHIFT+K	SHIFT+Command+K	neutralize acids and bases
CTRL+SHIFT+L	SHIFT+Command+L	move all water molecules and metals to ligand side
CTRL+SHIFT+M	SHIFT+Command+M	move all water molecules and metals to macromolecule side
CTRL+ALT+O	Option+Command+O	minimize the MMFF94 energy of the ligand
CTRL+ALT+G	Option+Command+G	generate ligand tautomers
CTRL+F9	Command+F9	create pharmacophore
SHIFT+F9	SHIFT+F9	create simplified pharmacophore (Catalyst)
CTRL+SHIFT+F9	SHIFT+Command+F9	create simplified pharmacophore (MOE)
CTRL+SHIFT+F10	SHIFT+Command+F10	create simplified pharmacophore (Phase)
CTRL+ALT+ SHIFT+F9	Option+SHIFT+Command+F9	create hotspot pharmacophore
CTRL+ALT+ SHIFT+P	Option+SHIFT+Command+P	clear pharmacophore
CTRL+SHIFT+NumPad+	SHIFT+Command++	increase selected feature tolerance by 0.15Å
CTRL+SHIFT+NumPad-	SHIFT+Command+-	decrease selected feature tolerance by 0.15Å
CTRL+ALT+C	Option+Command+C	center coordinates of all structures
CTRL+ALT+R	Option+Command+R	set reference
CTRL+ALT+X	Option+Command+X	clear all elements
CTRL+ALT+M	Option+Command+M	combine features in one pharmacophore
CTRL+ALT+T	Option+Command+T	interpolate all features within tolerances
CTRL+E	Command+E	interpolate features
CTRL+V	Command+V	simple rendering while moving
CTRL+L	Command+L	toggle background color (between black and white)
CTRL+Page Up	Command+Page Up	center camera on ligand
CTRL+Page Down	Command+Page Down	center camera on binding site
CTRL+ALT+SHIFT+W		ligand van der waals surface
CTRL+ALT+SHIFT+S		ligand solvent accessible surface

Windows/Linux	Mac OSX	Function
CTRL+ALT+SHIFT+M		ligand molecular surface
CTRL+ALT+SHIFT+B		surface of receptor binding pocket
CTRL+SHIFT+U	SHIFT+Command+U	recalculate & update surface
CTRL+2	Command+2	toggle 2D View
CTRL+T	Command+T	toggle Hierarchy View
CTRL+SHIFT+A	SHIFT+Command+A	show all views and tables
CTRL+SHIFT+V	SHIFT+Command+V	show 3D View only
F1	SHIFT+Command+H	user manual, tutorials, examples
1, 2, 3	1, 2, 3	set the specified bond type to the selected bond(s)
C, N, O, P, S	C, N, O, P, S	set the specified atom type to the selected atom(s)
Cursor-LEFT	Cursor-LEFT	left rotation in 3D viewer
Cursor-RIGHT	Cursor-RIGHT	right rotation in 3D viewer
Cursor-UP	Cursor-Up	up rotation in 3D viewer
Cursor-DOWN	Cursor-Down	down rotation in 3D viewer
CTRL+Cursor-LEFT	Command+Cursor-LEFT	left rotation in 3D viewer by 90 degrees
CTRL+Cursor-RIGHT	Command+Cursor-RIGHT	right rotation in 3D viewer by 90 degrees
CTRL+Cursor-UP	Command+Cursor-Up	up rotation in 3D viewer by 90 degrees
CTRL+Cursor-DOWN	Command+Cursor-Down	down rotation in 3D viewer by 90 degrees

A.4. LigandScout Mouse Bindings

A.4.1. All Views and Panels

Mouse Button	Activity	Effect
LEFT	click	Select single element
LEFT	double click	Extend selection to whole element; Macromolecule View : Select whole macromolecule; Active Site View : Select whole ligand, amino acid, or pharmacophore
SHIFT+LEFT	press	2D and 3D View: Box selection Hierarchy View, Select consecutive elements

Mouse Button	Activity	Effect
CTRL+LEFT	click	Expand selection: Select individual elements

A.4.2. 3D View

Mouse Button	Activity	Effect
LEFT	press	Rotate
LEFT	click	Select single element
MIDDLE/WHEEL	press or scroll	Zoom in or out
RIGHT	press	Translate

A.4.3. 3D Alignment Perspective

For the following interactions it is assumed that at least one element was highlighted (yellow).

Mouse Button	Activity	Effect
ALT+SHIFT+LEFT	press	Rotate, but alter coordinates of the selected elements
ALT+SHIFT+RIGHT	press	Translate, but alter coordinates of the selected elements

A.5. Command Line Usage

The generation of conformations, ligand-based pharmacophores and the screening of molecule libraries can be called from the command line. The executables are located in the LigandScout installation directory.

A.5.1. Conformation Generation

Usage:

`ldbgen` [Mandatory arguments] [Optional arguments] [Distribution arguments]

Mandatory arguments:

<code>--input, -i {infile}</code>	input file (*.sdf, *.smi, *.mol2, *.ldb)
-----------------------------------	--

--output, -o {outfile}	output file (*.ldb)
Optional arguments:	
--help, -h {type}	command line parameter help
type: all	lists all arguments
simple	shows most important arguments
distribution	shows network arguments
[param]	displays detailed help for the argument '[param]'
--log-file, -l {logfile}	output file for logging-messages (default: stdout)
--confgen-type, -t {type}	confgen type (default: omega-fast)
type: omega-fast	omega high-throughput settings (25 confs)
omega-best	omega high-quality settings (400 confs)
import	import from input file
--multi-conf, -m	auto-sense conformations in SDF files by name comparison (default: off)
--num-confs, -n {number}	max. number of conformations to generate (default set by '--confgentype' option)
--name-property, -p {prop-name}	property for overriding molecule name
--omega-license, -z {file}	omega license file (default: file specified by environment variable OE_LICENSE)
--error-file, -e {file}	file that collects molecules where errors occurred (default: {OUTPUT_FILE}-failed.log)
--update-keep-old, -K	update database and keep old entries
--update-drop-old, -R	update database and drop old entries
--allow-duplicates, -A	allow duplicates in the database (default: off)
--overwrite-existing, -O	overwrite already existing molecules (default: off)
--num-processes, -b {#processes}	number of processes to start (default: number of CPUs)
--exec-mode, -x {mode}	the execution mode (default: multi)
mode: single	only one CPU is used
multi	uses all CPUs/cores of this computer
network	uses computers in the network
Distribution options:	
--host-list-file, -H {file}	host list file (default: \${HOME}/.inteligand-data/ligand-scout3/iworker-list.txt)
--comma-sep-hosts, -c {list}	comma separated host list (HOST1:PORT1,HOST2:PORT2,...)

<code>--network-interface, -N {name}</code>	network interface to use ('-n list' to list all available interfaces)
<code>--verbosity, -v {loglevel}</code>	network logging verbosity (default: off)
loglevel: off	no network log messages
low	network errors and warnings
medium	connection info messages are shown
high	high-level protocol messages
highest	debug (very verbose)

A.5.2. Ligand-based Pharmacophore Generation

Usage:

`espresso` [Mandatory arguments] [Optional arguments]

Mandatory arguments:

<code>--training-set, -t {filename}</code>	input file containing the training-set (*.sdf, *.mol2, *.ldb, *.smi)
--	--

Optional arguments:

<code>--input, -i {filename}</code>	input file containing the training- and/or test-set (*.lsd)
<code>--help, -h {helptype}</code>	command line parameter help
helptype: all	lists all arguments
simple	shows most important arguments
[param]	displays detailed help for the argument '[param]'
<code>--quiet, -q</code>	suppress logging and info messages
<code>--log-file, -l {logfile}</code>	output file for logging-messages (default: stdout)
<code>--test-set, -s {file}</code>	input file containing the test-set (*.sdf, *.mol2, *.ldb, *.smi)
<code>--pharmacophore, -p {file}</code>	output file containing the generated pharmacophore (*.pmz, *.pml)
<code>--output, -o {file}</code>	output file for ligand conformations that were used for pharmacophore generation (*.sdf, *.mol2, *.ldb, *.smi, *.lsd)
<code>--confgen-type, -t {type}</code>	confgen type (default: omega-fast)
type: omega-fast	omega high-throughput settings (25 confs)
omega-best	omega high-quality settings (400 confs)
import	import from input file
<code>--num-confs, -n {number}</code>	max. number of conformations to generate (default set by '--confgentype' option)

<code>--omega-license, -z {file}</code>	omega license file (default: file specified by environment variable OE_LICENSE)
<code>--keep-confs, -y</code>	keep input conformations (default: override)
<code>--enable-excluded-volumes, -x</code>	generate exclusion volumes from training-set
<code>--pharmacophore-type, -g {type}</code>	the type of the pharmacophore to generate (default: merged)
type: shared	shared feature pharmacophore
merged	merged feature pharmacophore
<code>--scoring-function, -f {score}</code>	the function to use for scoring intermediate solutions and resulting pharmacophores (default: combo)
score: pharma_fit	scores the number of matching pharmacophore features and the RMSD of the pharmacophore alignment
fast_shape	scores the atom van der Waals sphere overlap of the aligned training-set molecules
shape	scores the molecular shape similarity of the aligned training-set molecules based on a gauss function representation of the molecular volume
combo	scores the number of matching pharmacophore features, the RMSD of the pharmacophore alignment and the atom van der Waals sphere overlap of the aligned training-set molecules
<code>--intermediate, -r {number}</code>	the number of intermediate alignment solutions to use (default: 10)
<code>--tolerance, -e {number}</code>	the global feature tolerance scaling factor (default: 1.0)
<code>--merged-omit, -m {number}</code>	max. omitted features for merged feature pharmacophores (default: 4)
<code>--mark-optional-features, -k</code>	mark partially matching features as optional (default: disabled)
<code>--optional-feature-t, -d {number}</code>	optional feature threshold (default: 10%)
<code>--pharm-name-prefix, -u {prefix}</code>	name-prefix for resulting pharmacophore(s) (default: Model)

A.5.3. Virtual Screening

Usage:

`iscreen` [Mandatory arguments] [Optional arguments] [Distribution arguments]

Mandatory arguments:

--query, -q {file}	query pharmacophore (*.pmz,*.pml)
--database, -d {file}	database to screen (*.ldb)
--output, -o {file}	hit list (*.sdf)

Optional arguments:

--help, -h {type}	command line parameter help
type: all	lists all arguments
simple	shows most important arguments
distribution	shows network arguments
[param]	displays detailed help for the argument '[param]'
--log-file, -l {logfile}	output file for logging-messages (default: stdout)
--disable-first-match-mode, -s	all matching conformations are considered (default: only first matching)
--disable-exclusion-volumes, -e	disable checking for exclusion volume clashes
--allow-omit, -a	maximum number of features to omit (default: 0)
--exec-mode, -x {mode}	the execution mode (default: multi)
mode: single	only one CPU is used
multi	uses all CPUs/cores of this computer
network	uses computers in the network

Distribution options:

--host-list-file, -H {file}	host list file (default: \${HOME}/.inteligand-data/ligand-scout3/iworker-list.txt)
--comma-sep-hosts, -c {list}	comma separated host list (HOST1:PORT1,HOST2:PORT2,...)
--network-interface, -N {name}	network interface to use ('-n list' to list all available interfaces)
--verbosity, -v {loglevel}	network logging verbosity (default: off)
loglevel: off	no network log messages
low	network errors and warnings
medium	connection info messages are shown
high	high-level protocol messages
highest	debug (very verbose)

A.5.4. Networking: iworker

Usage:

`iworker` [Optional arguments]

Optional arguments:

<code>--help, -h {helptype}</code>	command line parameter help
helptype: all	lists all arguments
simple	shows most important arguments
[param]	displays detailed help for the argument '[param]'
<code>--log-file, -l {logfile}</code>	output file for logging-messages (default: stdout)
<code>--auto-detection, -a</code>	enable auto-detection (default: off)
<code>--max-parallel-tasks, -m {NUMBER}</code>	maximum number of parallel executed tasks (default: number of CPUs)
<code>--network-interface, -N {name}</code>	network interface to use ('-n list' to list all available interfaces)
<code>--port, -p {NUMBER}</code>	tcp server port to use (default:30000)
<code>--verbosity, -v {loglevel}</code>	network logging verbosity (default: off)
loglevel: off	no network log messages
low	network errors and warnings
medium	connection info messages are shown
high	high-level protocol messages
highest	debug (very verbose)

A.6. Scoring Function

LigandScout provides several scoring functions for ranking intermediate solutions during the ligand-based pharmacophore generation. All scoring functions were developed to deliver robust results for several investigated targets. However, different scoring functions may deliver different results, depending on whether shape (i.e., molecular volume) or chemical functionality is considered to be more relevant for the assumed binding mode. Available scoring functions comprise Pharmacophore Fit (only taking into account chemical feature overlap), Atom Overlap ("Fast Shape"), Gaussian Shape Similarity, and a combination of Pharmacophore Fit and Atom Overlap. These scoring functions are described in detail below.

A.6.1. Simple Geometric Scoring Function (“Pharmacophore Fit”)

$$S_{RMS} = 9 - 3 * \min(RMS_{FP}, 3)$$

$$S_{FCR} = c * N_{MFP} + S_{RMS}$$

S_{FCR}	is the feature count/RMS distance score
S_{RMS}	is the matched feature pair RMS distance score in the range [0,9]
RMS_{FP}	is the RMS of the matched feature pair distances
N_{MFP}	is the number of geometrically matched feature pairs
c	is a weighting factor for the number of matched feature pairs (currently 10.0)

A.6.2. Atom Sphere Overlap (“Fast Shape”)

$$S_{MFC} = \frac{N_{MFP}}{\min(NF_1, NF_2)}$$

S_{ARO}	is the atom radius overlap score in the range [0,1]
n	is total number of atoms of all involved molecules
V_{imax}	is the maximum pseudo overlap volume of the i th atom with an atom of another involved molecule and lies in the range [0,1]. For the overlap volume to become non-zero, the atom has to overlap with at least one atom from each other involved molecule
S_{ARO}	is the atom radius overlap score in the range [0,1]

A.6.3. Gaussian shape similarity

$$S_{GSO} = \frac{V_i}{\sum_i V_{SOi} - V_i}$$

S_{GSO}	is the gauss shape overlap similarity in the range [0,1]
V_i	is the common volume of intersection of all involved molecules
V_{SOi}	is volume of intersection of the i th molecule with itself

A.6.4. Pharmacophore Fit and Atom Overlap

$$S_{MFC} = \frac{N_{MFP}}{\min(NF_1, NF_2)}$$

$$S_{FD} = \frac{\sum_{i=1}^n d_i}{N_{MFP}}$$

$$S_{RMS} = 1 - \frac{\min(RMS_{FP}, RMS_{max})}{RMS_{max}}$$

$$S_{DOF} = S_{ARO} * 0.3 + S_{MFC} * 0.3 + S_{FD} * 0.3 + S_{RMS} * 0.1$$

S_{DOF}	is the pharmacophore fit and atom overlap score
S_{MFC}	is the matched feature pair score
N_{MFP}	is the number of geometrically matched feature pairs
NF_1	is the number of features in the first pharmacophore
NF_2	is the number of features in the second pharmacophore
S_{FD}	is the matched feature pair distance score
d_i	is the distance of the i th matched feature pair if the distance is greater than 1, and zero otherwise
S_{RMS}	is the matched feature pair RMS distance score [0,1]
RMS_{max}	is the maximum RMS to consider (set to 5.0)
RMS_{FP}	is the RMS of the matched feature pair distances
S_{ARO}	is the atom radius overlap score in the range [0,1]

A.7. Primary Literature

Please visit our website for an [up-to-date list of publications on LigandScout](http://www.inteligand.com/ligandscout/pubs.shtml) [http://www.inteligand.com/ligandscout/pubs.shtml].

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