



## Creating a pharmacophore from a single protein-ligand complex

Experience level: basic  
Time needed: 5 minutes

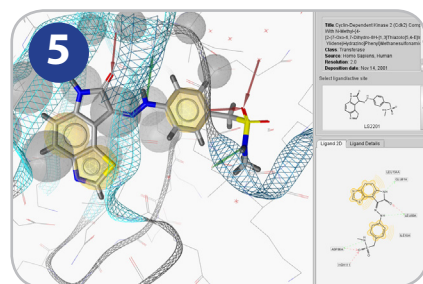
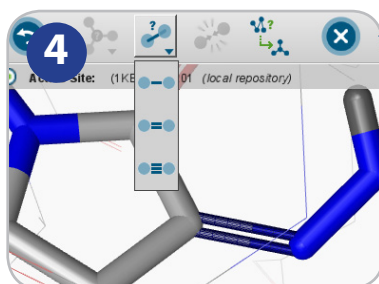
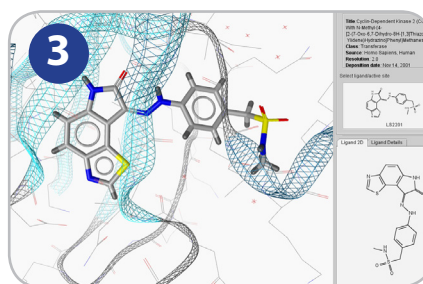
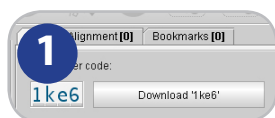
Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecule view</li> <li>Active site view</li> </ul>	<ul style="list-style-type: none"> <li>Download PDB file using 4-letter code</li> <li>Discover the correct ligand</li> <li>Focus on active site and switch to active site view</li> <li>Check and eventually correct ligand chemistry</li> <li>Create a structure-based pharmacophore</li> </ul>	<ul style="list-style-type: none"> <li>Ligand box</li> <li>Create pharmacophore (button or menu)</li> <li>OR: Create pharmacophore for Catalyst/MOE/Phase</li> </ul>	<ul style="list-style-type: none"> <li>Change bond type</li> <li>Change atom type</li> <li>Create new bond</li> <li>Move to core</li> <li>Move to environment</li> <li>Compound switching</li> </ul>

### Description:

Type '1ke6' in the upper right area of the screen and press the button 'Download 1ke6' [1]. The protein will be downloaded and displayed (this is the 'macromolecule view') [2]. Click on the yellow box within the protein representing the ligand - an animated zoom begins ending in [3]. Since ligand structures in the PDB only contain incomplete information, you should always check whether all bonds are typed correctly. Bond types can be changed by selecting a bond (by clicking on it either in the 2D or 3D view) and by using the retype bond button [5] or the keys 1, 2, or 3. Once the ligand is chemically correct, create a pharmacophore by pressing Ctrl-F9 (Cmd-F9 on OS X) [4].

### Where to go from here:

- Pharmacophore modeling: Creating shared and merged feature pharmacophores
- Customizing pharmacophore creation preferences
- Virtual screening





## Pharmacophore modeling: Create shared & merged feature pharmacophores using chemical features from three PDB structures

Experience level: medium

Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecule view</li> <li>Active site view</li> <li>Alignment view</li> </ul>	<ul style="list-style-type: none"> <li>Download PDB File '1ke6'</li> <li>Check &amp; correct ligand</li> <li>Create pharmacophore</li> <li>Add ligand and pharmacophore to alignment view</li> <li>Repeat the last 4 steps for '1ke7' and '1ke8'</li> <li>Create a 'shared feature pharmacophore' from the 3 pharmacophores</li> <li>Align the three ligands to the new pharmacophore</li> </ul>	<ul style="list-style-type: none"> <li>Ligand box</li> <li>Create pharmacophore (button or menu)</li> <li>Add molecule to alignment</li> <li>Add pharmacophore to alignment</li> <li>Generate shared feature pharmacophore (by features)</li> <li>Generate merged feature pharmacophore</li> </ul>	<ul style="list-style-type: none"> <li>Set reference element</li> <li>Center all structures</li> <li>Alignment context menu</li> </ul>

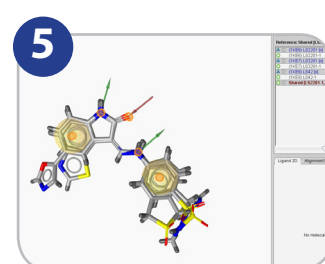
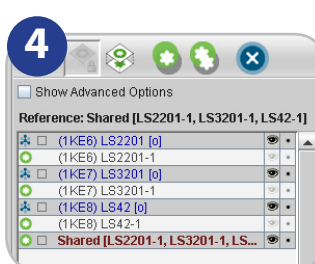
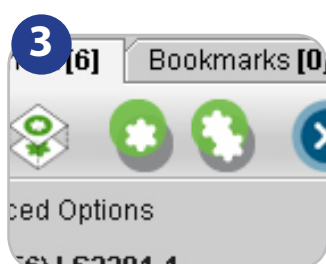
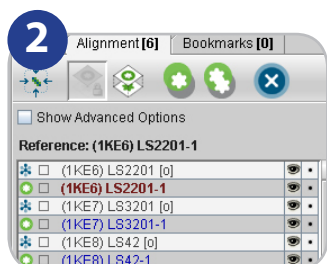
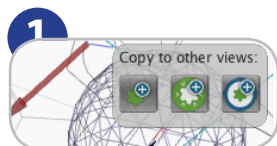
### Description:

Type '1ke6' in the upper right area of the screen and press the button 'Download '1ke6''. The protein will be downloaded and displayed in macromolecule view. Click on the yellow box to focus on ligand 'LS2201'. Check the ligand for its correctness. Press ctrl-f9 to create a pharmacophore (cmd-f9 on OS X). Use the data exchange widget and select 'add molecule to alignment view' [1a] and the molecule will be added to the alignment view, increasing a counter in the corresponding tab. Use the data exchange widget and select 'add pharmacophore to alignment view' [1b] to do the same with the pharmacophore. Repeat these steps with '1ke7', and '1ke8'. Finally, you should have six elements in the alignment view. Click on the 'Alignment' tab to work with them.

Select the three pharmacophores in the alignment list using Ctrl-click or multiple selection [2], and press the 'Generate shared feature pharmacophore (by features)' button [3]. A new pharmacophore called 'Shared [LS2201-1, LS3201-1, LS42-1]' is appended to the list. Mark this element and make it the reference element using the 'Set reference' button, which will change the display style of this element to red and bold. Now use the ctrl key (cmd on OS X) to additionally select the molecules identified by the blue icons [4]. Additionally, hide the three pharmacophores derived from 1ke6, 1ke7, and 1ke8 by clicking on the eye symbols on the right next to these alignment entries [4]. Now press the 'align' button. You will see the three ligands aligned to the shared feature pharmacophore derived from the three PDB complexes [5].

### Where to go from here:

- Customizing pharmacophore creation preferences
- Virtual screening in LigandScout





## Virtual screening and comparing active/inactive hit retrieval rates

Experience level: advanced

Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecule view</li> <li>Active site view</li> </ul>	<ul style="list-style-type: none"> <li>Create pharmacophore for virtual screening</li> <li>Initiate virtual screening</li> <li>Refine pharmacophore model</li> <li>Repeat virtual screening</li> </ul>	<ul style="list-style-type: none"> <li>Ligand box</li> <li>Create pharmacophore (button or menu)</li> <li>Initiate virtual screening</li> </ul>	<ul style="list-style-type: none"> <li>Virtual screening with omitted features</li> </ul>

### Description:

Virtual screening aims at the maximum enrichment of active compounds in a hit list. Therefore, such methods are usually validated by assessing the accuracy of discrimination between actives and decoys. A good pharmacophore model will be able to identify a significant portion of known active compounds and as few decoys as possible. In this tutorial, two different types of precalculated multiconformational databases are available: A library containing a selection of known active compounds and a library of true unactives (here used as decoys).

Type '1ke7' in the upper right area of the screen, press the button 'Download 1ke7'. Create a pharmacophore of the protein-ligand complex as described in the LigandScout tutorial sheet 1 'Creating a pharmacophore from a single protein-ligand complex' [1]. Perform virtual screening using a multi-conformational database with known active compounds (filename 'CDK2-Actives.ldb') for screening using the 'Screen pharmacophore against external library' item located in the 'Pharmacophore' menu [2]. Virtual screening will be initiated after selecting the library file (press the button with the three dots to open a file dialog) and the number of omissible features [3].

Since the initial pharmacophore was derived from all the interactions of only one single ligand, it is rather restrictive and retrieves only few hits. Downsize the number of features by deleting less important features [4] or increase the tolerances of the features by selecting a feature and then choosing 'Pharmacophore' -> 'Increase Selected Feature Tolerance' from the menu. Compare the ratio of active compounds and decoys (library filename 'CDK2-Inactives.ldb') retrieved by your pharmacophore in order to estimate its predictive power [5]. Further adapt the pharmacophore in order to improve the ratio of actives and decoys obtained by virtual screening: Remove non-essential chemical features and exclusion volume spheres in order to increase sensitivity; adapt the size of the feature spheres by pressing 'ctrl-shift-plus' or the corresponding menu entry ('Pharmacophore - Decrease Selected Feature Tolerance') or use the pharmacophore from tutorial card 2 using the data exchange widgets [6].

### Where to go from here:

- Pharmacophore modeling: Creating shared and merged feature pharmacophores
- Customizing pharmacophore creation preferences
- Analyzing screening results

The collage consists of six numbered screenshots:

- 1**: A button labeled 'Screening [4]' with a green star icon.
- 2**: A menu titled 'Create pharmacophore' with options: 'Clear Pharmacophore', 'Create Exclusion Volumes Coat', 'Create Exclusion Volume on Selected Atoms', 'Increase Selected Feature Tolerance by 0.15Å', 'Decrease Selected Feature Tolerance by 0.15Å', and 'Screen Pharmacophore Against External Library'.
- 3**: A 'Screening Settings' dialog box showing 'Scoring Function: Pharmacophore Fit', 'Max. Num. omitted Features: 0', a checked box for 'Stop after first matching Conformation', and 'Screened Database: /db/xdb\_files/cdk'.
- 4**: A 3D molecular model showing a ligand (yellow sticks) bound to a protein (grey surface), with a pharmacophore model overlaid as a yellow mesh.
- 5**: A table of search results with columns: Name, fit/Value, time, and n. The table lists several ZINC compounds and their corresponding fit values and times.
- 6**: A 3D molecular model showing a ligand (red sticks) bound to a protein (grey surface), with a pharmacophore model overlaid as a red mesh. A 'Copy to other views:' button is visible.



## Analyze LigandScout pharmacophore screening results

Experience level: medium

Time needed: 10 minutes

Prerequisites: Virtual screening hit list

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Library view (spread-sheet)</li> <li>Hierarchy view</li> </ul>	<ul style="list-style-type: none"> <li>Create or import screening results</li> <li>Sort virtual screening hit list</li> <li>Inspect and compare hits</li> <li>Discover controls of the library view</li> </ul>	<ul style="list-style-type: none"> <li>Library view (Spread-sheet)</li> <li>Viewer controls</li> </ul>	<ul style="list-style-type: none"> <li>Calculation of Gaussian Shape Similarity Score</li> </ul>

### Description:

Perform virtual screening to obtain a hit list as described on tutorial card 4 'Virtual screening and comparing active/inactive hit retrieval rates' or insert external screening results from a library file via the 'File - Insert' menu entry. Results of virtual screening are depicted in the library view [1]. Sort the hit list by a single click on the 'Pharm. Fit Score' table header [2]. You may invert the sorting order by an additional click. Select a compound of interest by single click on the corresponding table row to be depicted in the active site view together with the original ligand [3]. Multiple compounds are selected when holding shift or ctrl (cmd on MAC OS X) while clicking. Keep compounds visible independent from the current selection state by clicking the eye symbol in the table left to the molecule name in the library view spreadsheet [4].

The visibility of the core molecule (i.e., the original ligand) and other items in the active site view is toggled with the eye symbol located in the hierarchy view on the right side next to the molecule name [5]. Select a custom color for the core molecule using the square icon next to the eye icon in the hierarchy view.

Calculation of the Gaussian Shape Similarity Score offers further functionality for scoring. Use the 'Calculate Gaussian Shape Similarity Score' function located in the 'Library' menu for calculation. A new property 'Shape Tanimoto Score' will be added in the library view and you can use the spreadsheet functionality for sorting and ranking as described above.

### Where to go from here:

- Pharmacophore modeling: Creating shared and merged feature pharmacophores
- Customizing pharmacophore creation preferences

**1**

Name	fitValue ▾	time	numConfs
03815609 [# 33]	87.62126	0.562	400
03815605 [# 32]	87.38204	0.656	400
04631147 [# 103]	78.38429	1.578	400
04631144 [# 104]	78.313644	2.765	400
03815598 [# 23]	78.28976	1.125	400
03815603 [# 26]	78.23573	0.796	196
03815607 [# 29]	78.14391	0.36	174
04631169 [# 112]	78.14371	1.235	219
03815608 [# 31]	78.121765	0.422	361
01550575 [# 2]	78.12091	0.672	400
04631092 [# 87]	78.11591	4.219	400
04631183 [# 118]	78.09796	1.547	400
03815602 [# 30]	78.07209	3.516	400
04631154 [# 106]	78.03461	0.375	400
04631177 [# 115]	78.02224	2.297	400
02047781 [# 9]	78.00544	0.735	400
03941407 [# 43]	78.00391	0.344	400
02007314 [# 7]	77.95323	3.954	400

**2**

Pharmacophore fit Score	
...	36.43
...	36.13
...	36.95
...	35.40
...	36.06
...	36.75
...	35.40
...	36.88

**3**

**4**

Name	
<input checked="" type="checkbox"/> ZINC03815609 [# 33]	
<input checked="" type="checkbox"/> ZINC03815605 [# 32]	
<input checked="" type="checkbox"/> ZINC04631147 [# 103]	
<input checked="" type="checkbox"/> ZINC04631144 [# 104]	
<input checked="" type="checkbox"/> ZINC03815598 [# 23]	
<input checked="" type="checkbox"/> ZINC03815603 [# 26]	

**5**





## View and rescore docking poses with pharmacophores

Experience level: medium

Time needed: 10 minutes

Prerequisites: docking poses from external sources

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Library view (spreadsheet)</li> <li>Hierarchy view</li> </ul>	<ul style="list-style-type: none"> <li>Download PDB File</li> <li>Switch to active site view</li> <li>Import docking poses</li> <li>Analyze docking poses</li> <li>Rescore docking poses</li> <li>Align docking poses to pharmacophore</li> <li>Discover the viewer controls of the library view</li> </ul>	<ul style="list-style-type: none"> <li>Library view (Spreadsheet)</li> <li>Viewer controls</li> </ul>	

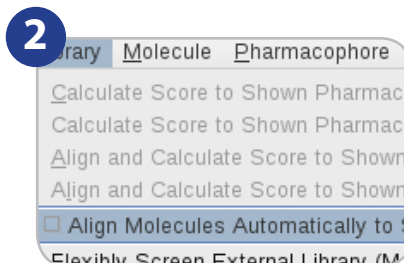
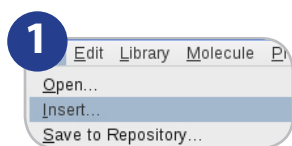
### Description:

Download '1ke7' and create the pharmacophore of the protein-ligand complex. Import the corresponding docking poses stored in MDL SDF file format (tutorial filename '1ke7-docking.sdf') via the 'File' - 'Insert' menu entry [1]. Make sure that the 'Align Molecules Automatically to Shown Pharmacophore' option located in the 'Library' menu is deactivated [2], which ensures that the coordinates of the docking poses relative to the protein are preserved. The compounds are imported to LigandScout including all properties given in the SD file, such as the docking score. Sort the list of docking poses by a single click on the table header corresponding to the docking score [3]. Invert the sorting order by an additional click if necessary.

LigandScout offers four different options for rescoring docking poses based on the 3D pharmacophore that is currently visible in LigandScout: The calculation of the pharmacophore fit of imported docking poses considering or ignoring excluded volume spheres, and the calculation of the pharmacophore fit of the docking poses after alignment to the pharmacophore considering or ignoring excluded volume spheres. Alignment to the pharmacophore before scoring may be useful to optimize docking poses [4]. All four modes are accessed via the 'Library' menu. You can modify the pharmacophore by deleting features, adding excluded volumes, etc. to reflect the important features of the binding site that should be fulfilled by the highest ranked hits. Alignment of imported docking poses to the shown pharmacophore is performed automatically upon selection of a spreadsheet line (i.e. a single docking pose) if the 'Align Molecules Automatically to Shown Pharmacophore' option located in the 'Library' menu is activated. Poses may also be aligned to the pharmacophore using LigandScout's alignment algorithm using the 'Align' button located in the upper right corner of the library view [5].

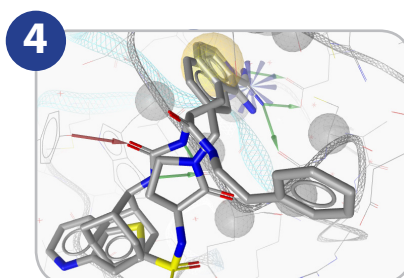
### Where to go from here:

- Customizing pharmacophore creation preferences



**3**

	Gold Chemscore	Fitness
s.omegasdf\16\dock2...	38.1147	7.966
s.omegasdf\136\dock...	37.1573	8.373
ldock2 (# 1)	35.5703	7.674
s.omegasdf\97\dock3...	34.9029	8.140
ldock1 (# 2)	34.7940	8.172
s.omegasdf\44\dock2...	34.7397	7.913
s.omegasdf\10\dock3...	34.7303	8.525
s.omegasdf\33\dock1...	33.9775	8.438
ldock7 (# 3)	33.9036	8.030
s.omegasdf\61\dock2...	33.7200	7.500
s.omegasdf\12\dock2...	33.5666	8.263
s.omegasdf\126\dock...	33.3315	8.26



**5**

Protein Rotated Atoms	Gold Chemscore Metal Weights
6 18.8885 H	0 0 0 0 0 0 0
1 18.9268 H	0 0 0 0 0 0 0
17.3050 H	0 0 0 0 0 0 0



## Espresso: Create ligand-based 3D pharmacophores

Experience level: intermediate

Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>○ Ligand-Based perspective</li> </ul>	<ul style="list-style-type: none"> <li>○ Import ligands</li> <li>○ Cluster ligand-set in 3D</li> <li>○ Generate ligand-based pharmacophore model</li> </ul>	<ul style="list-style-type: none"> <li>○ 3D Clustering</li> <li>○ Create ligand-based pharmacophore (button or menu)</li> </ul>	<ul style="list-style-type: none"> <li>○ Use different settings for conformational model generation</li> <li>○ Use different settings for clustering</li> <li>○ Use different settings for ligand-based pharmacophore generation</li> </ul>

### Description:

Open the smiles file containing your ligands ('CDK2-Actives.smi') by choosing the 'File- Open' menu. The ligands are imported and shown in 2D on the right side of the screen [1]. Set all candidates for the model to 'training set' by using the green button of the 'Type' field [2], or choosing 'Ligand-Set -> Flag Selected Molecules -> As Training Set'. Cluster the ligand set according to their 3D pharmacophore characteristics using the 'Cluster' button on the bottom of the 3D view [3-Yellow]. Keep the default setting in the following dialog and start the clustering process by pressing the 'OK' button. Espresso will create two new columns in the ligand table called 'Cluster ID' and 'Cluster Size'. Set all molecules to 'ignored' with the dedicated button [3-Blue]. Sort then the table by clicking on the column header 'Cluster Size'. Then set the compounds with the highest cluster size value -and having the same cluster ID- to training set. To do this, select a molecule of the cluster you want to use and press the 'Set Selected Cluster ID to training' button [3-Gray]. Now click on 'Create ligand-based pharmacophore' [3-Red] using the default values to start pharmacophore generation. A ligand-based model will be created and displayed in the 3D view. Feature patterns [4] in the table indicate which features are met by which ligand. A click on a colored square indicates which feature is linked to the corresponding square. The list on the right lower corner of the screen shows several pharmacophore solutions. By selecting a different solution, it is shown in the 3D view with the ligands aligned accordingly [5]. You can use the data exchange controls on the upper right corner of the 3D view to move the currently shown pharmacophore to other views (e.g. Screening) or save it using the 'File -> Save as File...' selecting a file type that represents pharmacophores.

### Where to go from here:

- Align structure-based pharmacophore models with ligand-based ones
- Use a pre-existing pharmacophore as bias during the ligand-based pharmacophore generation process
- Virtual screening

**1** Three chemical structures of ligands are shown side-by-side.

**2** A 'Type' dropdown menu is shown with a green button.

**3** A toolbar is shown with a yellow 'Cluster' button.

**4** A 'Matching Features' table is shown with colored squares.

**5** A 3D view is shown with ligands aligned with a pharmacophore model.



## Screen several databases using the “Screening Perspective”

Experience level: intermediate  
Time needed: 5 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Screening view</li> </ul>	<ul style="list-style-type: none"> <li>Add pharmacophore model to screening view</li> <li>Load virtual database</li> <li>Perform virtual screening</li> </ul>	<ul style="list-style-type: none"> <li>Load Screening Database (Button)</li> <li>Perform Screening (Button)</li> </ul>	<ul style="list-style-type: none"> <li>none</li> </ul>

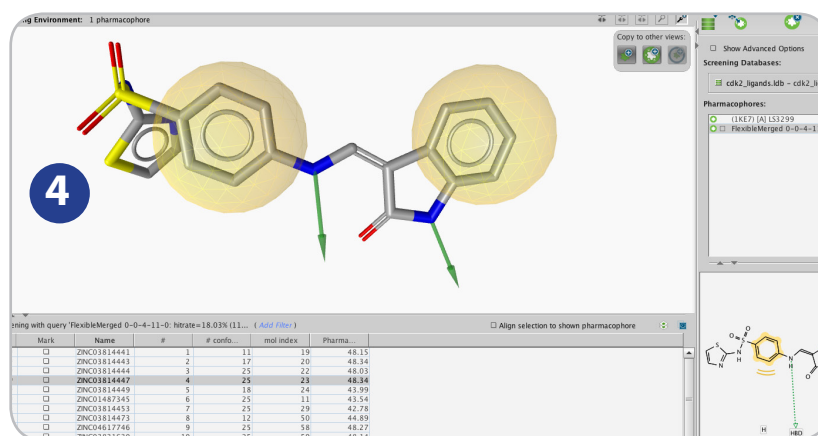
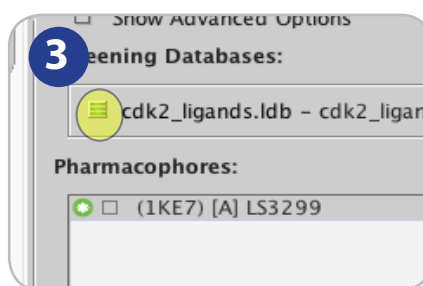
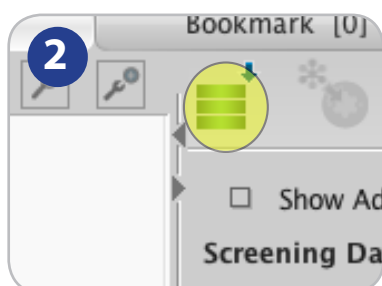
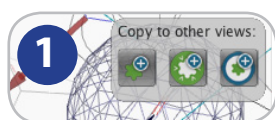
### Description:

In contrast to the virtual screening procedure in the ‘Structure-based Modeling’ perspective which is designed for pharmacophore model refinement with respect to ligand-binding pocket properties, the ‘Screening’ perspective provides a fast and convenient way of performing multiple virtual screens with different screening databases against your final pharmacophore model, which can be useful for validation.

Import your favorite pharmacophore model (e.g. the one created from tutorial card 1 or 6) into the ‘Screening’ perspective either by using the ‘Exchange Data Widget’ [1] or the ‘Open’ entry in the ‘File’ menu. Click on the ‘Load Screening Database’ button [2] to load the screening database ‘cdk2-ligands.ldb’. Enable the loaded screening database for screening by clicking on the grey button left to the screening database entry [3]. Now, select the pharmacophore model from the ‘Pharmacophores’ list to make sure it is shown in the 3D view. Next to the ‘Load Screening Database’ button, the ‘Perform Screening’ and ‘Delete Pharmacophore’ button will be enabled. Click on the ‘Perform Screening’ button to initiate the virtual screening. View the results in the table at the bottom of the screen [4]. You can add more databases and pharmacophores for further virtual screening experiments. Please make sure that only one pharmacophore is visible before you start screening.

### Where to go from here:

- Analyze screening results
- Advanced screening: ROC curves and model combination





## Advanced screening: ROC curves and model combination

Experience level: advanced  
Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Screening view</li> </ul>	<ul style="list-style-type: none"> <li>Add pharmacophore models to screening view</li> <li>Load virtual database</li> <li>Perform virtual screening</li> <li>Plot ROC curve</li> <li>Combine models by 'OR'</li> </ul>	<ul style="list-style-type: none"> <li>Load Screening Database (Button)</li> <li>Perform Screening (Button)</li> <li>Plot ROC Curve (Button)</li> </ul>	<ul style="list-style-type: none"> <li>Boolean Expression (Field)</li> </ul>

### Description:

In contrast to the virtual screening procedure in the 'Structure-based Modeling' perspective which is designed for pharmacophore model refinement with respect to ligand-binding pocket properties, the 'Screening' perspective provides means for statistical evaluation of screening performance.

Import the structure-based pharmacophore models from PDB entry 1ke7 (tutorial card 1) and the ligand-based CDK2 pharmacophore (tutorial card 2) into the 'Screening' perspective either by using the 'Exchange Data Widget' [1] or, alternatively, load a saved version using the 'Open' entry in the 'File' menu. Click on the 'Load Screening Database' button [2] to load the screening database 'CDK2-Actives.ldb', and click the same button again and load the database 'CDK2-Inactives.ldb'. Click once on the tri-state selector left to the database names to mark 'cdk2-ligands.ldb' as a database containing actives (green database symbol), and twice on the tri-state selector left of 'CDK2-Inactives.ldb' to mark this database as decoys (red database symbol) [3]. Now, select the pharmacophore model '1ke7' from the 'Pharmacophores' list to make sure it is shown in the 3D view. Click on the 'Perform Screening' button to initiate the virtual screening [4]. After screening has finished, press the 'Plot ROC Curve' button [5] to plot a ROC curve [6].

Now select both pharmacophores in the 'Pharmacophores' table. Numbers in curly brackets ("{1}" and "{2}") indicate the number of the pharmacophore. Enter "1 or 2" into the boolean expression field [7] indicating that a virtual hit in this screening run should either fit into the first (1) or to the second (2) pharmacophore. Press the screening button [4] again to re-screen, and the ROC curve button [5] again to compare the virtual screening statistics.

View the virtual hits in the table at the bottom of the screen [8]. You can add more databases and pharmacophores for further virtual screening experiments.

### Where to go from here:

- Analyze screening results

The collage consists of eight numbered screenshots:

- 1**: A screenshot of the 'Copy to other views' dialog box, showing options to copy data to the 3D, 2D, and Table views.
- 2**: A screenshot of the 'Load Screening Database' button in the software interface.
- 3**: A screenshot of the 'cdk2\_ligands.ldb - cdk2' and 'cdk2\_decoys.ldb - cdk2' entries in the database list, with the tri-state selectors being manipulated.
- 4**: A screenshot of the 'Perform Screening' button in the software interface.
- 5**: A screenshot of the 'Plot ROC Curve' button in the software interface.
- 6**: A screenshot of the ROC curve plot. The y-axis is 'Sensitivity (% selected ligands)' and the x-axis is '1 - Specificity (% selected decoys)'. The plot shows a solid blue line above a dashed diagonal line. Text on the plot indicates: '92 hits of 2073 total compounds (58 actives, 2015 decoys) AUC: 0.57 EF1/5/10: 12.5 / 3.9 / 3.9'.
- 7**: A screenshot of the 'Pharmacophores' table. It shows two entries: '(1KE7) [A] LS3299' and 'FlexibleMerged 1-4-19-6-1...'. The '1 or 2' boolean expression is entered in the field below.
- 8**: A screenshot of the 3D view showing a chemical structure of a ligand bound to a protein, with a table of results at the bottom.



## LigandScout Molecule Tables: Filtering & Export

Experience level: intermediate

Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>○ IDBGEN Database generator</li> <li>○ Structure-based view</li> <li>○ Table view</li> </ul>	<ul style="list-style-type: none"> <li>○ Create a multiconformational database from a smiles file</li> <li>○ Examine the compounds</li> <li>○ Calculate physicochemical properties</li> <li>○ Filter and export molecular structures</li> </ul>	<ul style="list-style-type: none"> <li>○ IDBGEN GUI</li> <li>○ Load LDB file</li> <li>○ Calculate standard properties</li> <li>○ Apply filter rules</li> </ul>	<ul style="list-style-type: none"> <li>○ Export 2D structure files</li> <li>○ Export to Microsoft Excel</li> </ul>

### Description:

Start the IDBGEN GUI molecular database generator [1]. Chose a smiles file of molecules as input file and specify the output file. Set the iCon FAST option for conformation generation [2]. After completion, load the resulting ldb file into the structure-based perspective and focus on the table view [3]. Calculate some physicochemical parameters and molecular descriptors using *Library -> Calculate Standard Properties*. Apply filters [4, 5] and view the dynamically generated results.

### Where to go from here:

- Export the molecular structures together with the calculated standard properties to a 2D SDF file
- Export the filtered compound list to a Microsoft Excel file

The screenshots show the following steps:

- 1**: IDBGEN GUI - Database generator. The 'Input File' field is set to 'smiles.txt' and the 'Output File' field is set to 'output.ldb'. The 'iCon FAST' option is selected under 'Conformation generation'.
- 2**: IDBGEN GUI - Database generator. The 'iCon FAST' option is selected under 'Conformation generation'.
- 3**: Structure-based perspective. The 'Table view' is selected, showing a table of compounds with columns: Mark, Name, #, # Confs, and MolWt. The table contains 9 rows of data.
- 4**: Filter dialog. The 'Filter' button is clicked, showing a list of properties to filter by: Name, mol index, # Confs, Mol. Index, MolWt, TPSA [Ertl], # Heavy Atoms, # Acceptors, # Rot. Bonds, cLogP, # Rings, # Donors, and Rel. TPSA.
- 5**: Filter results. The 'Filter' button is clicked, showing a list of properties to filter by: Name, mol index, # Confs, Mol. Index, MolWt, TPSA [Ertl], # Heavy Atoms, # Acceptors, # Rot. Bonds, cLogP, # Rings, # Donors, and Rel. TPSA.





## In Silico Fragment Screening with LigandScout

Experience level: advanced

Time needed: 15 minutes

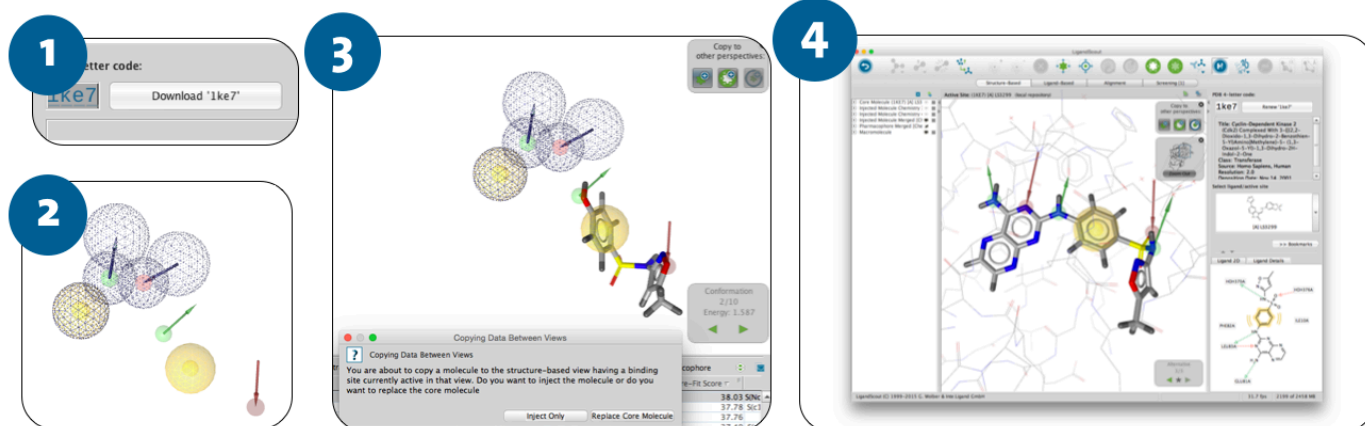
Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecular view</li> <li>Screening view</li> <li>Active site view</li> </ul>	<ul style="list-style-type: none"> <li>Create structure based pharmacophore model</li> <li>Switch to screening view</li> <li>Perform fragment screening</li> <li>Inject hits back into protein binding site</li> <li>Join fragments to create new molecules</li> </ul>	<ul style="list-style-type: none"> <li>Ligand box</li> <li>Create pharmacophore</li> <li>Set features optional</li> <li>Initiate virtual screening</li> <li>Library view</li> <li>Inject hits into protein binding site</li> <li>Minimize hits</li> <li>Merge molecules</li> </ul>	<ul style="list-style-type: none"> <li>Re-dock resulting molecules and minimize</li> <li>Re-score the molecules by estimating the free energy of binding</li> <li>Export results</li> </ul>

### Description:

Type “1ke7” in the upper right area of the screen, press the button “Download 1ke7” [1], click on the yellow box, and generate a pharmacophore model. Move the model to the Screening view. Load the tutorial fragment library (fragments4tutorial.ldb). Set the left three features as optional by selecting them (press Shift+left mouse button and draw a rectangle around them) and use the *Pharmacophore -> Mark Features as Optional* command [2]. Perform screening, examine the resulting hits, and inject the most suitable ones back into the protein binding site [3]. Reset the three left features as mandatory using the *Pharmacophore -> Unmark Features as Optional* command, set the right two features as optional and repeat the screening. Examine the resulting hits, and inject the most suitable ones back into the protein binding site. In the Structure-based perspective, minimize each fragment and observe their behavior. Using the viewer option in the hierarchical view, make two fragments visible that overlap with one atom. Delete one of the overlapping atoms and merge the molecules using the *Molecule -> Merge Visible Molecules* command. Create a bond between both fragments. Minimize the resulting new molecule and create a pharmacophore model. Analyze the interactions and compare them to the model generated from the initial ligand. Repeat this procedure for other fragment combinations [4].

### Where to go from here:

- Re-dock, minimize, and re-score newly created molecules in order to identify the most interesting candidates
- Export the most favorable designs as an Excel spreadsheet, or as 3D or 2D sdf files





## Finding druggable pockets in proteins with LigandScout

Experience level: advanced

Time needed: 10 minutes

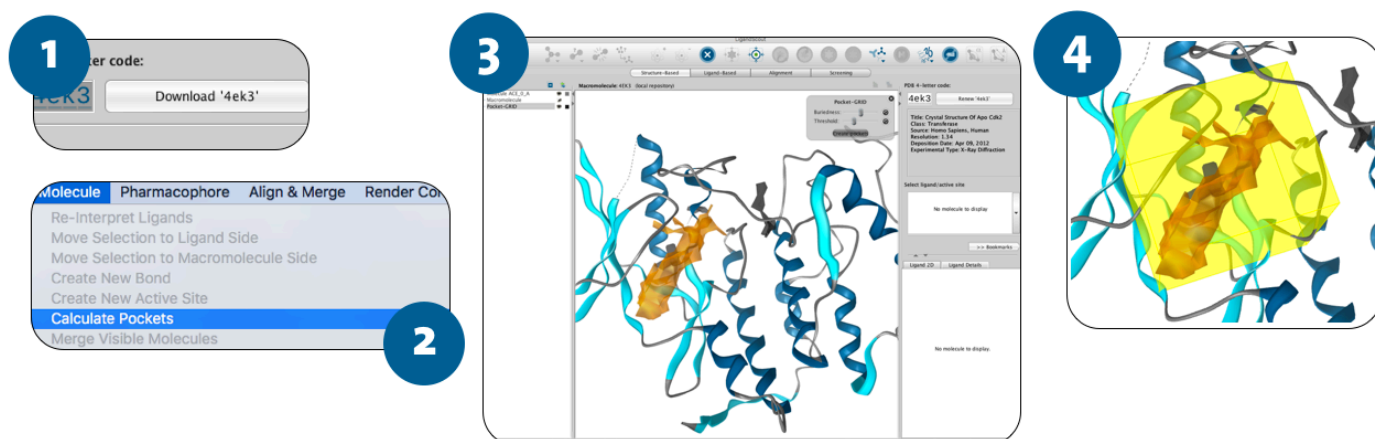
Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecular view</li> <li>Active site view</li> </ul>	<ul style="list-style-type: none"> <li>Download PDB file using 4-letter code</li> <li>Perform pocket analysis</li> <li>Select and create a new binding pocket</li> </ul>	<ul style="list-style-type: none"> <li>Pocket finder widget</li> <li>Ligand box</li> </ul>	<ul style="list-style-type: none"> <li>Adjust parameters for buriedness and threshold</li> <li>Create multiple binding pockets</li> <li>Perform docking</li> <li>Perform apo-site pharmacophore model generation</li> </ul>

### Description:

Type “4eq3” in the upper right area of the screen and press the button “Download”[1]. From the dropdown menu, select *Molecule -> Calculate Pockets* [2]. A grid is calculated over the entire protein structure and grid points are evaluated according to their buriedness and their number of neighboring grid points. Isocontour surfaces are generated [3]: Orange colored isosurfaces are considered to highlight druggable pockets, where dark grey colored ones are considered too small to accommodate a drug-like ligand. Values for buriedness and threshold can be modified by moving the sliders on the Pocket-GRID widget [3], while clicking on the *Create pockets* button will define a new binding site highlighted by the yellow box [4]. Clicking on the yellow box will zoom into the Active site view and from here, docking or apo-site pharmacophore creation can be performed.

### Where to go from here:

- Dock ligands into the newly defined binding site
- Create apo-site pharmacophore models





## Creating pharmacophore models in empty binding sites

Experience level: advanced

Time needed: 25 minutes

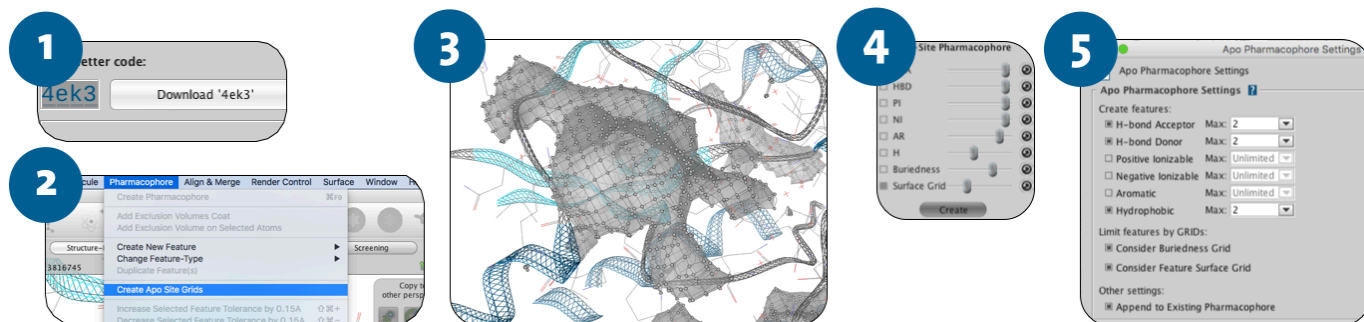
Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecule view</li> <li>Active site view</li> <li>Hierarchical view</li> </ul>	<ul style="list-style-type: none"> <li>Open PDB file</li> <li>Find potential binding pocket</li> <li>Switch to active site view</li> <li>Create apo site grids</li> <li>Create apo site pharmacophore model</li> </ul>	<ul style="list-style-type: none"> <li>Pocket finder widget</li> <li>Ligand box</li> <li>Ligand accessible surface property display</li> <li>Define apo site pharmacophore settings</li> </ul>	<ul style="list-style-type: none"> <li>Perform virtual screening</li> <li>Refine pharmacophore models from retrieved hit molecules</li> <li>Move pharmacophore features in 3D space</li> </ul>

### Description:

Type “4ek3” in the upper right area of the screen, and press the button “Download 4ek3” [1]. The protein will be downloaded and displayed in the macromolecule view. Search for the druggable pocket using the LigandScout Pocket Finder (see tutorial card 11) and define the binding site. Zoom into it by clicking on the yellow box. Calculate the entire possible pharmacophore interactions by selecting *Pharmacophore* -> *Create Apo Site Grids* [2]. Modify the apo site pharmacophore feature probabilities by moving the sliders in the Apo-Site Pharmacophore widget [4]: Moving sliders to the right will make features less probable, moving them to the left will make features more probable. Restore default settings by clicking the *return back* symbol located at the right of the slider. By clicking the *Create* button, the *Apo-Pharmacophore Settings* dialogue will appear [5]. Create a model by selecting the nature and number of features that you want to be considered in the automated generation process. Start with HBA, HBD, and hydrophobic features. Repeat the procedure for positive and negative ionizable features. Refine the model by modifying the final number of features, merging some of them, or moving them to new positions. Select grid points and add features if necessary. Add or remove exclusion volume spheres. Use the model for virtual screening or for re-scoring docked poses.

### Where to go from here:

- Insert hits obtained from VS into the binding site
- Create new pharmacophore models from minimized hit structures obtained
- Re-score poses obtained from molecular docking





## Docking a molecule into a binding site using the built-in AutoDock program

Experience level: intermediate

Time needed: 25 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>Macromolecule view</li> <li>Active site view</li> <li>Table view</li> </ul>	<ul style="list-style-type: none"> <li>Download PDB file using 4-letter code</li> <li>Switch to active site view</li> <li>Insert molecule into the binding site</li> <li>Perform the docking experiment</li> </ul>	<ul style="list-style-type: none"> <li>Ligand box</li> <li>Insert smiles string</li> <li>Dock molecule</li> <li>Analyze docking poses</li> </ul>	<ul style="list-style-type: none"> <li>Filter poses based on pharmacophore features</li> <li>Minimize poses</li> <li>Re-score poses by estimating the free energy of binding</li> <li>Create new binding site</li> <li>Dock molecules into new binding site</li> </ul>

### Description:

Type “1ke7” in the upper right area of the screen, and press the button “Download 1ke7” [1]. The protein will be downloaded and displayed in the macromolecule view. Click on the yellow box within the protein representing the ligand in order to zoom into the binding site. Insert the molecules as sdf that you want to dock into the binding site, using *File -> Insert* [2] or by pasting a smiles file (*Cntrl-V*). Select the molecules that you want to dock from the table below the 3D window. Proceed with the docking experiment by using *Molecule -> Dock Ligands -> AutoDock 4.2 or AutoDock Vina 1.1* [3]. After the experiment is finished, the results will be displayed in the table view [4], each compound ranked according to the most favorable interaction energy. Analyze the docked poses by creating pharmacophore models for viewing the interactions with the protein. Filter the poses based on the interactions common to those of the original ligand.

### Where to go from here:

- Minimize the docked poses
- Calculate the free energy of binding and determine the MMFF94 estimated binding enthalpies
- Calculate the pharmacophore fit score based on the model generated from the original ligand structure
- Create a new binding site by selecting amino acids with the mouse (hold left button while pressing the “Shift” key, move over the region you want to investigate and create a binding site from the menu *Molecule -> Create New Binding Site*), or use the pocket finder (see tutorial card 11) for finding a new druggable pocket and repeat the docking experiment.

The sequence of screenshots shows the following steps:

- Step 1:** The 'Download 1ke7' button is highlighted in the top right corner of the interface.
- Step 2:** The 'File' menu is open, and the 'Insert...' option is highlighted.
- Step 3:** The 'Molecule' menu is open, and the 'Dock Ligands (AutoDock 4.2)' option is highlighted.
- Step 4:** The 'Table' view is displayed, showing a list of docked ligands with columns for Name, MMFF94 Binding Energy, and RMSD. The first entry is highlighted.



## Analyzing Molecular Dynamics Trajectories with LigandScout

Experience level: advanced

Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
<ul style="list-style-type: none"> <li>○ Macromolecule view</li> <li>○ Active site view</li> </ul>	<ul style="list-style-type: none"> <li>○ Open PDB file</li> <li>○ Switch to active site view</li> <li>○ Load molecular dynamics trajectory file</li> <li>○ Analyze the trajectory</li> <li>○ Create pharmacophore models</li> </ul>	<ul style="list-style-type: none"> <li>○ Ligand box</li> <li>○ Molecular dynamics trajectory Widget</li> <li>○ Playback trajectory</li> <li>○ Create pharmacophore models</li> <li>○ Align pharmacophore models</li> </ul>	<ul style="list-style-type: none"> <li>○ Create shared feature models</li> <li>○ Perform virtual screening with MD derived pharmacophore models</li> </ul>

### Description:

Load the PDB file containing the initial frame of your molecular dynamics simulation (1ke7\_start.pdb) [1]. Click on the yellow box within the protein representing the ligand in order to zoom into the binding site. Load the DCD file of the molecular dynamics trajectory (1ke7\_trajectories.dcd) [2] and select the trajectory in the hierarchical view [3]. Press the play button in the MD trajectory widget [4] for viewing the results of the dynamics simulations [5]. Pause at any time and generate a pharmacophore model. Move the pharmacophore model into the alignment perspective. Repeat this several times. In the alignment perspective, analyze the features that are common in all models [6].

### Where to go from here:

- Align pharmacophore models generated from MD trajectory
- Create shared feature and merged feature models from the different pharmacophore families
- Perform virtual screening with the different models

