

# Ligand Scout Tutorials

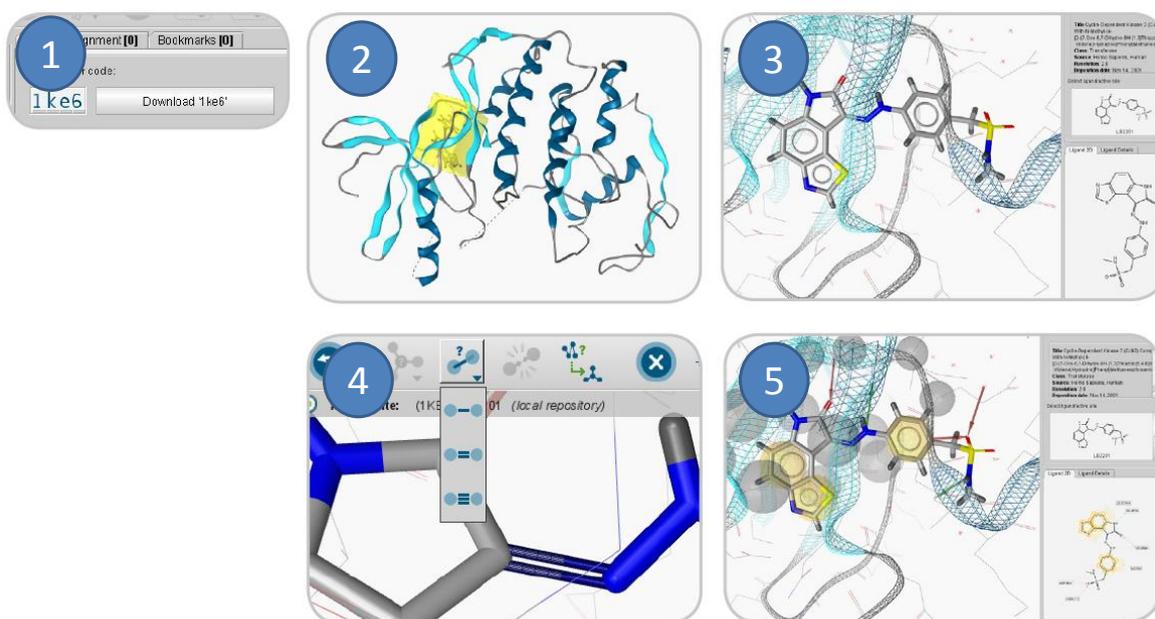
## Step 1: Creating a pharmacophore from a protein-ligand complex.

Type '1ke6' in the upper right area of the screen and press the button 'Download' [1]. The protein will be downloaded and displayed in the *Structure-Based* view.

Click on the yellow box within the protein to focus on ligand 'LS2201' [2] -> [3].

Since ligand structures in the PDB only contain incomplete information (especially for bond orders), 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 [4] or the keys 1, 2, or 3 of the numeric pad.

Once the ligand is chemically correct, create a pharmacophore by selecting *Pharmacophore* > *Create Pharmacophore* or by pressing Ctrl-F9 [5].



## **Step 2: Create consensus pharmacophores using 3 PDB structures.**

Use the data exchange widget, click on the 'Copy current ligand(s) to' button and choose *Alignment Perspective*[1a]. The molecule will be added to the alignment view.

Use again the data exchange widget and select 'Copy current pharmacophore(s) to' button and choose *Alignment Perspective*[1b].

Repeat these steps with '1ke7', and '1ke8'. Finally, there are six elements in the alignment view.

Click on the 'Alignment' tab (on bottom) to work with them. Select the three pharmacophores in the alignment list using Ctrl-click identified by the green icons, and press the 'Generate shared feature pharmacophore (by features)' button [2a].

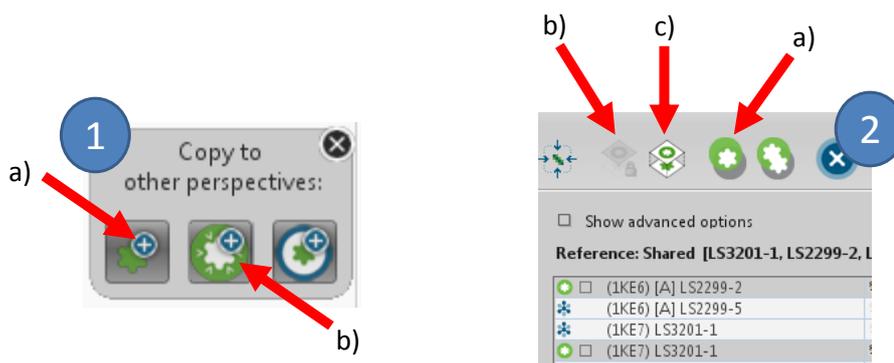
A new pharmacophore called 'Shared [LS2201-1, LS3201-1, LS42-1]' is appended to the list. Click on this element and make it the reference element using the 'Set reference' button [2b].

Use the ctrl key to additionally select the molecules identified by the blue icons.

Hide the three pharmacophores derived from 1ke6, 1ke7, and 1ke8 by clicking on the eye symbols on the right next to these alignment entries.

Press the 'align' button [2c].

Then the three ligands are aligned to the shared feature pharmacophore derived from the three PDB complexes.



### Step 3: Virtual screening and comparing active/inactive hit rates.

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 conformers databases are available: A library containing a selection of known active compounds and a library of decoys.

Type '1ke7' in the upper right area of the screen then press the button 'Download'.

Create a pharmacophore of the protein-ligand complex as described in the Ligand Scout tutorial 1.

Perform virtual screening using a multi-conformational database with known active compounds (filename 'cdk2-ligands.ldb') for screening using the 'Screen pharmacophore against external library' item located in the 'Pharmacophore' menu [1]. 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 [2].

Compare the ratio of active compounds and decoys (library filename 'cdk2-decoys.ldb') retrieved by the pharmacophore in order to estimate its predictive power [3].

Adapt the pharmacophore [4] 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').

Use the pharmacophore from tutorial card 2 using the data exchange widgets [5].

1

2

3

4

5

Mark	Name	#	mol index
1	ZINC0003491	10	1

## **Step 4: Analyze LigandScout pharmacophore screening results.**

Perform virtual screening to obtain a hit list as described on step 3 'Virtual screening and comparing active/inactive hit retrieval rates' or insert external screening results from a library file via the 'File - Insert' menu entry.

Sort the hit list by a single click on the 'Pharmacophore fit Score' table header [1]. 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 [2]. Multiple compounds are selected when holding shift or ctrl 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 [3].

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 [4]. 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 'Gaussian Shape Similarity Score' function located in the 'Library' menu for calculation [5]. A new property 'Gaussian Shape Similarity Score' will be added in the library view and you can use the spreadsheet functionality for sorting and ranking as described above.

**1**

	Pharmacophore fit Score ▲
16	64.52
33	64.80
18	65.10
50	65.30

**2**

**3**

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input checked="" type="checkbox"/>
4	<input type="checkbox"/>
5	<input type="checkbox"/>
6	<input type="checkbox"/>
7	<input type="checkbox"/>

**4**

Core Molecule (1KE7) [A] LS3299	<input type="checkbox"/>	<input type="checkbox"/>
[-] Injected Library Molecule ZINC03814444	<input type="checkbox"/>	<input type="checkbox"/>
[-] Injected Library Molecule ZINC03831630	<input type="checkbox"/>	<input type="checkbox"/>
[-] Injected Library Molecule ZINC04617747	<input type="checkbox"/>	<input type="checkbox"/>

**5**

Library	Ligand-Set	Molecule	Pharm
Calculate Score to Shown Pharmacophore			
Calculate Score to Shown Pharmacophore (			
Align and Calculate Score to Shown Pharma			
Align and Calculate Score to Shown Pharma			
Calculate Standard Properties			
Calculate Gaussian Shape Similarity Score			
Calculate MMFF94 Strain Energy			

## **Step 5: View and rescore docking poses with pharmacophores.**

Download '1ke7' and create the pharmacophore of the protein-ligand complex as in step 1.

Import the docking poses stored in MDL SDF file format (filename '1ke7\_ligands\_docking.sdf') via the 'File' - 'Insert' menu entry.

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 decreasing values by a single click on the table header corresponding to the docking score 'Gold.Goldscore.Fitness' [1].

There are four different options accessible via the 'Library' menu for rescoring docking poses based on the 3D pharmacophore that is currently visible in LigandScout [2]:

- The calculation of the pharmacophore fit of imported docking poses considering/ignoring excluded volume spheres.
- The calculation of the pharmacophore fit of the docking poses *after alignment to the pharmacophore* considering/ignoring excluded volume spheres. (Alignment to the pharmacophore before scoring may be useful to optimize docking poses.)

Compare the results from these 4 possible scoring options. Scores are shown under the table header of type 'Pharm. Score'.

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.

1

Gold.Goldscore.Fitness ▾
68.3137
67.9871
66.7460
66.6223
66.3517
65.9001

2

Library	Ligand-Set	Molecule	Pharmacophore	Align & Merge	Render Cor
Calculate Score to Shown Pharmacophore					
Calculate Score to Shown Pharmacophore (Without Exclusion Volumes)					
Align and Calculate Score to Shown Pharmacophore					
Align and Calculate Score to Shown Pharmacophore (Without Exclusion Volumes)					

## Step 6: Espresso: Create ligand-based 3D pharmacophores.

Open the smiles file containing your ligands ('cdk2.smi') by choosing the 'File-Open' menu. Click on the 'Ligand-Based' tab (on bottom) to work with them. The ligands are shown in 2D on the right side of the screen [1].

Set all candidates for the model to 'training set' by 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 [2a] or via the 'Ligand-Set' menu > Cluster Ligand-Set. Keep the default setting in the following dialog and start the clustering process by pressing the 'OK' button. A new column in the ligand table called 'Cluster ID' is created.

Sort the table using this new column by clicking on the column header 'Cluster ID'. Set all molecules with cluster ID '2' to test set by using the table controls [3].

Now click on the button 'Create ligand-based pharmacophore' [2b] 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].

Save the currently shown pharmacophore using the 'File -> Save' menu and selecting a file type that represents pharmacophores.

Type
7501 Training
6101 Training
42 Test
11 Test
2201 Test
3201 Training
51 Test
Ignored

name	score
Model-1	0.8830
Model-2	0.8816
Model-3	0.8761
Model-4	0.8733
Model-5	0.8715

## **Step7: Screen several databases using the “Screening Perspective”.**

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 permits to perform multiple virtual screens with different screening databases against a final pharmacophore model, which can be useful for validation.

Import the structure-based pharmacophore models from PDB entry 1ke6 (step 1) and 1ke7 (step2) 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 [2a] to load the screening database 'cdk2-ligands.ldb'. Do the same for the database 'cdk2-decoys.ldb'.

Click once on the grey button left to the database names to mark 'cdk2-ligands.ldb' as a database containing actives (green database symbol), and twice on the grey button left of 'cdk2-decoys.ldb' to mark this database as decoys (red database symbol) [3].

Select the pharmacophore model '1ke6' 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.

Now select pharmacophores 1ke6 and 1ke7 in the 'Pharmacophore

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 again to re-screen, and compare the virtual screening statistics.

