



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

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

The figure shows five numbered steps in the LigandScout interface:

- Step 1:** The search bar contains '1ke6' and the 'Download 1ke6' button is highlighted.
- Step 2:** The protein structure is displayed in a ribbon representation with a yellow box highlighting the ligand binding site.
- Step 3:** The view is zoomed into the active site, showing the ligand (1KE6) in stick representation within a mesh surface.
- Step 4:** The 'Active site: (1KE6)' panel is open, showing the 'Pharmacophore' button and a 'Local repository'.
- Step 5:** The pharmacophore is generated and displayed as a yellow mesh surface over the ligand, with a 'Retype bond' button visible in the interface.



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

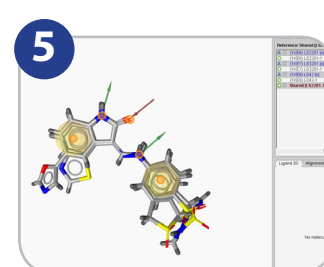
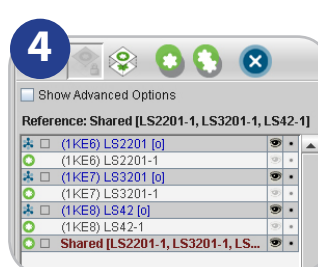
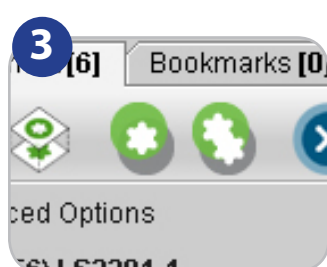
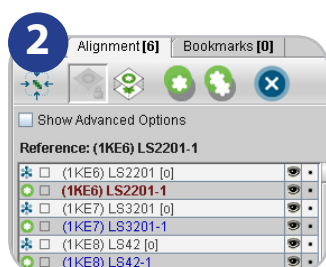
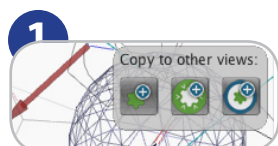
Description:

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"> Macromolecule view Active site view 	<ul style="list-style-type: none"> Create pharmacophore for virtual screening Initiate virtual screening Refine pharmacophore model Repeat virtual screening 	<ul style="list-style-type: none"> Ligand box Create pharmacophore (button or menu) Initiate virtual screening 	<ul style="list-style-type: none"> Virtual screening with omitted features

Description:

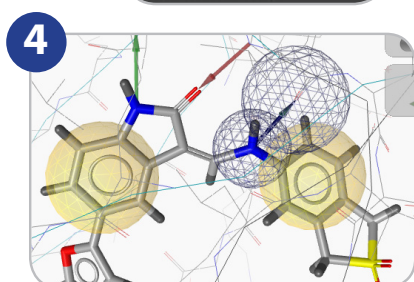
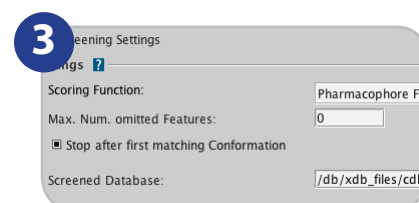
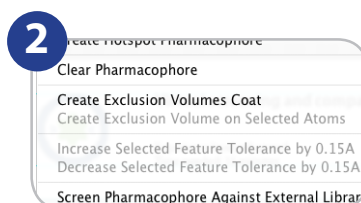
Virtual screening aims at the maximum enrichment of active compounds in a hit list. Therefore, such methods are usually validated by assessing the accuracy of discrimination between actives and decoys. A good pharmacophore model will be able to identify a significant portion of known active compounds 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 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-ligands.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].

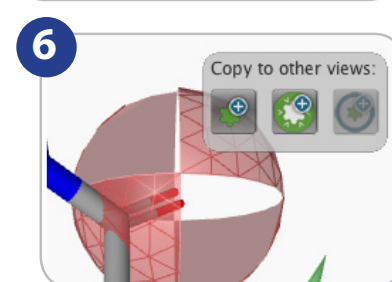
Since the initial pharmacophore was derived from all the interactions of only one single ligand, it is rather restrictive and retrieves only few hits. Downsize the number of features by deleting less important features [4] or increase the tolerances of the features by selecting a feature and then choosing 'Pharmacophore' -> 'Increase Selected Feature Tolerance' from the menu. Compare the ratio of active compounds and decoys (library filename 'cdk2-decoys.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



Name	fitValue	time	n
ZINC03815609 [# 33]	87.62126	0.562	
ZINC03815605 [# 32]	87.38204	0.656	
ZINC04631147 [# 103]	78.38429	1.578	
ZINC04631144 [# 104]	78.313644	2.765	
ZINC03815598 [# 23]	78.28976	1.125	
ZINC03815603 [# 26]	78.23573	0.796	
ZINC03815607 [# 29]	78.14391	0.36	
ZINC04631169 [# 112]	78.14371	1.235	
ZINC03815608 [# 31]	78.121765	0.422	
ZINC01550575 [# 2]	78.12091	0.672	
ZINC04631092 [# 87]	78.11591	4.219	
ZINC04631183 [# 118]	78.09796	1.547	
ZINC03815602 [# 30]	78.07209	3.516	
ZINC04631154 [# 106]	78.02461	0.275	





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"> Library view (spread-sheet) Hierarchy view 	<ul style="list-style-type: none"> Create or import screening results Sort virtual screening hit list Inspect and compare hits Discover controls of the library view 	<ul style="list-style-type: none"> Library view (Spread-sheet) Viewer controls 	<ul style="list-style-type: none"> Calculation of shape tanimoto score

Description:

Perform virtual screening to obtain a hit list as described on tutorial card 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. Results of virtual screening are depicted in the library view [1]. Sort the hit list by a single click on the 'FitValue' 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 '+' 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 Shape Tanimoto score offers further functionality for scoring. Use the 'Calculate Shape Tanimoto 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
- Performing virtual screening

1 Screenshot of the library view spreadsheet showing columns: Name, fitValue, time, numConfs. The first few rows are highlighted.

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
0007314 [# 7]	77.95323	3.954	400

2 Screenshot of the library view spreadsheet with 'fitValue' selected as the sorting criterion. The rows are sorted by fitValue in descending order.

	fitValue
# 33]	87.62126
# 32]	87.38204
# 103]	78.38429
# 104]	78.313644
# 23]	78.28976

3 Screenshot of the active site view showing the original ligand (blue) and a selected hit (yellow) docked in the binding pocket.

4 Screenshot of the library view spreadsheet with the 'Name' column selected. The first few rows are highlighted, showing the '+' symbol next to the molecule names.

Name
ZINC03815609 [# 33]
ZINC03815605 [# 32]
ZINC04631147 [# 103]
ZINC04631144 [# 104]

5 Screenshot of the hierarchy view showing the molecule name 'ecule ZINC0' and a list of eye icons for toggling visibility. The first eye icon is highlighted.



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

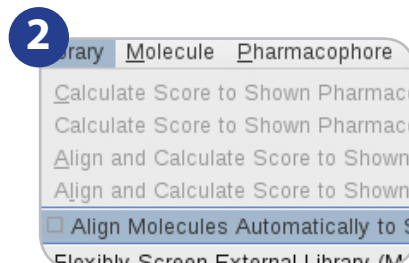
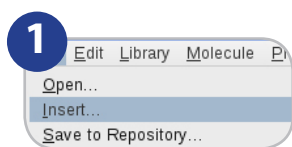
Description:

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_ligands_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].

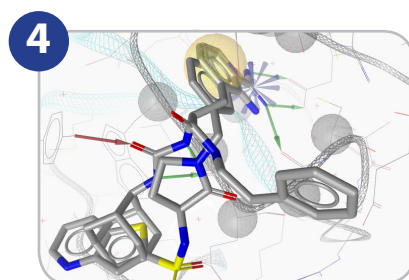
Where to go from here:

- Customizing pharmacophore creation preferences



3

	Gold_Chemscore.Fitness	r
s.omegasdf\16\dock2...	38.1147	7.966
s.omegasdf\136\dock...	37.1573	8.3734
ldock2 [#1]	35.5703	7.674
s.omegasdf\97\dock3...	34.9029	8.1405
ldock1 [#2]	34.7940	8.1723
s.omegasdf\44\dock2...	34.7397	7.9134
s.omegasdf\10\dock3...	34.7303	8.5255
s.omegasdf\33\dock1...	33.9775	8.4385
ldock7 [#3]	33.9036	8.0303
s.omegasdf\61\dock2...	33.7200	7.5005
s.omegasdf\12\dock2...	33.5666	8.2633
s.omegasdf\126\dock...	33.3315	8.20...



5

	Gold_Chemscore.Metal.Weight
6 18.8885 H 0 0 0 0 0 0 0 0	
7 18.9268 H 0 0 0 0 0 0 0 0	
17.3050 H 0 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"> Ligand-based Modeling 	<ul style="list-style-type: none"> Import ligands Cluster ligand-set in 3D Generate ligand-based pharmacophore model 	<ul style="list-style-type: none"> 3D Clustering Generate ligand-based pharmacophore model (button or menu) 	<ul style="list-style-type: none"> Use different settings for conformational model generation Use different settings for clustering Use different settings for ligand-based pharmacophore model generation

Description:

Open the smiles file containing your ligands ('cdk2.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 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 [2]. Keep the default setting in the following dialog and start the clustering process by pressing the 'OK' button. Espresso will create a new column in the ligand table called 'Cluster ID'. Sort the table using this column by clicking on the column header 'Cluster ID'. Set all molecules with cluster ID '1' to test set by using the table controls [3]. Now click on the button 'Create ligand-based pharmacophore' [4] using the default values to start pharmacophore generation. A ligand-based model will be created and displayed in the 3D view. Feature patterns [5] 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 [6]. 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' menu and selecting a file type that represents pharmacophores.

Where to go from here:

- Align structure-based pharmacophore models with ligand-based ones
- Virtual screening in LigandScout

The figure consists of six numbered screenshots illustrating the Espresso workflow:

- 1**: A table of imported ligands (L551, L53201, L542) shown in 2D chemical structure.
- 2**: The 3D view interface with the 'Cluster' button highlighted.
- 3**: A dialog box for setting molecule status, with 'Training' selected for the current molecule.
- 4**: The 'Create ligand-based pharmacophore' button in the 3D view.
- 5**: A 'Feature Pattern' table showing which features are present for each ligand, indicated by colored squares.
- 6**: The 3D view showing multiple pharmacophore models overlaid on the ligands.



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"> Screening view 	<ul style="list-style-type: none"> Add pharmacophore model to screening view Load virtual database Perform virtual screening 	<ul style="list-style-type: none"> Load Screening Database (Button) Perform Screening (Button) 	<ul style="list-style-type: none"> none

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

The screenshots illustrate the workflow:

- Step 1:** Clicking the 'Copy to other views' button to load the pharmacophore model into the screening view.
- Step 2:** Clicking the 'Load Screening Database' button to load the 'cdk2_ligands.ldb' database.
- Step 3:** Enabling the 'cdk2_ligands.ldb' database and selecting the '(1KE7) [A] LS3299' pharmacophore model.
- Step 4:** Viewing the 3D model and the resulting screening results table.

Mark	Name	#	# confs.	mol index	Pharma.
<input type="checkbox"/>	ZNCO1814441	1	11	19	48.15
<input type="checkbox"/>	ZNCO1814443	2	17	20	48.34
<input type="checkbox"/>	ZNCO1814444	3	25	22	48.03
<input type="checkbox"/>	ZNCO1814447	4	25	28	48.84
<input type="checkbox"/>	ZNCO1814449	5	18	24	43.99
<input type="checkbox"/>	ZNCO18144745	6	25	11	43.54
<input type="checkbox"/>	ZNCO1814453	7	25	29	42.78
<input type="checkbox"/>	ZNCO1814473	8	12	50	44.89
<input type="checkbox"/>	ZNCO4617746	9	25	38	48.27
<input type="checkbox"/>	ZNCO18181630	10	25	58	48.14



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"> Screening view 	<ul style="list-style-type: none"> Add pharmacophore models to screening view Load virtual database Perform virtual screening Plot ROC curve Combine models by 'OR' 	<ul style="list-style-type: none"> Load Screening Database (Button) Perform Screening (Button) Plot ROC Curve (Button) 	<ul style="list-style-type: none"> Boolean Expression (Field)

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-ligands.ldb', and click the same button again and load the database 'cdk2-decoys.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-decoys.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

1 Copy to other views: Screenshot of the 'Exchange Data Widget' showing a 3D molecular model and a 'Copy to other views' button.

2 Screenshot of the 'Load Screening Database' button in the software interface.

3 Screenshot of the 'Pharmacophores' list showing 'cdk2_ligands.ldb - cdk2' and 'cdk2_decoys.ldb - cdk2' with tri-state selectors.

4 Screenshot of the 'Perform Screening' button.

5 Screenshot of the 'Plot ROC Curve' button.

6 ROC curve plot showing Sensitivity (% selected ligands) vs 1 - Specificity (% selected decoys). The plot shows a blue curve above a dashed diagonal line. Text on the plot: 92 hits of 2073 total compounds (58 actives, 2015 decoys), AUC: 0.57, EF1/5/10: 12.5 / 3.9 / 3.9.

7 Screenshot of the 'Pharmacophores' table with '1ke7' and 'FlexibleMerged 1-4-19-6-1...' selected, and the boolean expression field set to '1 or 2'.

8 Screenshot of the 3D molecular model view showing a ligand bound to a protein, with a table of results below it.

ID	Name	#	# actives	hit ratio	Priority
1	2m2114411	2	20	20	49.19
2	2m2114412	2	20	20	49.19
3	2m2114413	2	20	20	49.19
4	2m2114414	2	20	20	49.19
5	2m2114415	2	20	20	49.19
6	2m2114416	2	20	20	49.19
7	2m2114417	2	20	20	49.19
8	2m2114418	2	20	20	49.19
9	2m2114419	2	20	20	49.19
10	2m2114420	2	20	20	49.19