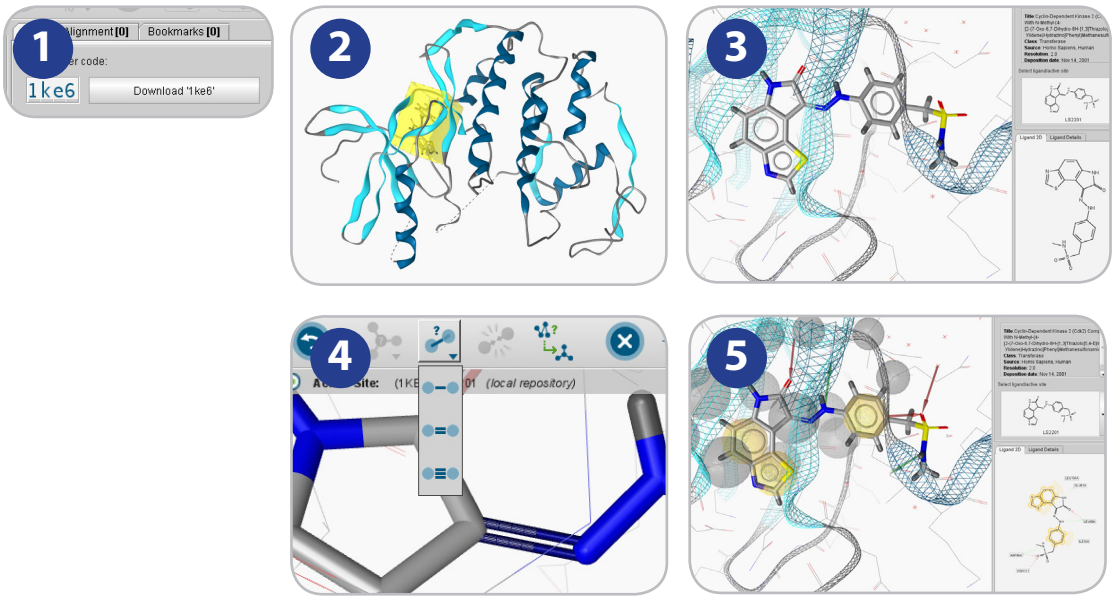
Creating a pharmacophore from a single protein-ligand complex

Experience level: basic
Time needed: 5 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
○ Macromolecule view ○ Active site view	○ 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	○ Ligand box ○ Create pharmacophore (button or menu) ○ OR: Create pharmacophore for Catalyst/MOE/Phase	○ 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



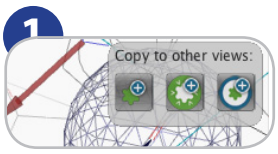
Pharmacophore modeling: Create shared & merged feature pharmacophores using chemical features from three PDB structures

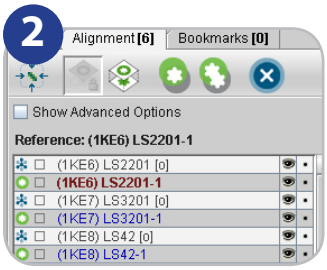
Experience level: medium
Time needed: 15 minutes

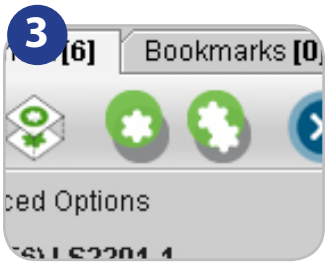
Views	Sequence	User Controls	Advanced controls (opt.)
○ Macromolecule view ○ Active site view ○ Alignment view	○ Download PDB File '1ke6' ○ Check & correct ligand ○ Create pharmacophore ○ Add ligand and pharmacophore to alignment view ○ Repeat the last 4 steps for '1ke7' and '1ke8' ○ Create a 'shared feature pharmacophore' from the 3 pharmacophores ○ Align the three ligands to the new pharmacophore	○ 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	○ Set reference element ○ Center all structures ○ Alignment context menu

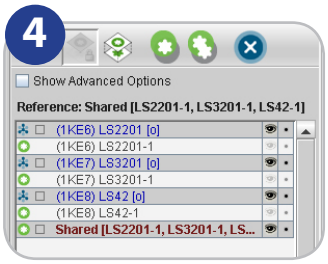
Description:
Type '1ke6' in the upper right area of the screen and press the button 'Download '1ke6'. The protein will be downloaded and displayed in macromolecule view. Click on the yellow box to focus on ligand 'LS2201'. Check the ligand for its correctness. Press ctrl-f9 to create a pharmacophore (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].

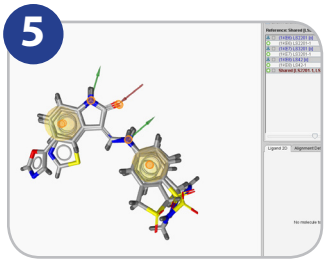
Where to go from here:
- Customizing pharmacophore creation preferences
- Virtual screening in LigandScout











Virtual screening and comparing active/inactive hit retrieval rates

Experience level: advanced
Time needed: 15 minutes

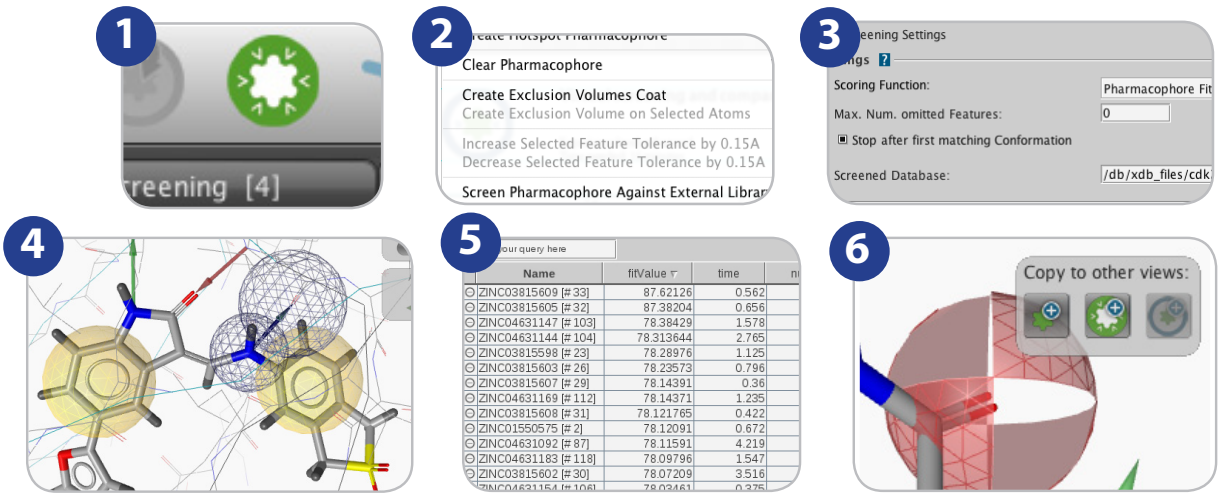
Views	Sequence	User Controls	Advanced controls (opt.)
○ Macromolecule view ○ Active site view	○ Create pharmacophore for virtual screening ○ Initiate virtual screening ○ Refine pharmacophore model ○ Repeat virtual screening	○ Ligand box ○ Create pharmacophore (button or menu) ○ Initiate virtual screening	○ 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 butten with the three dots to open a file dialog) and the number of omittable 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



Analyze LigandScout pharmacophore screening results

Experience level: medium
Time needed: 10 minutes
Prerequisites: Virtual screening hit list

Views	Sequence	User Controls	Advanced controls (opt.)
○ Library view (spread-sheet) ○ Hierarchy view	○ Create or import screening results ○ Sort virtual screening hit list ○ Inspect and compare hits ○ Discover controls of the library view	○ Library view (Spread-sheet) ○ Viewer controls	○ Calculation of Gaussion Shape Similarity Score

Description:

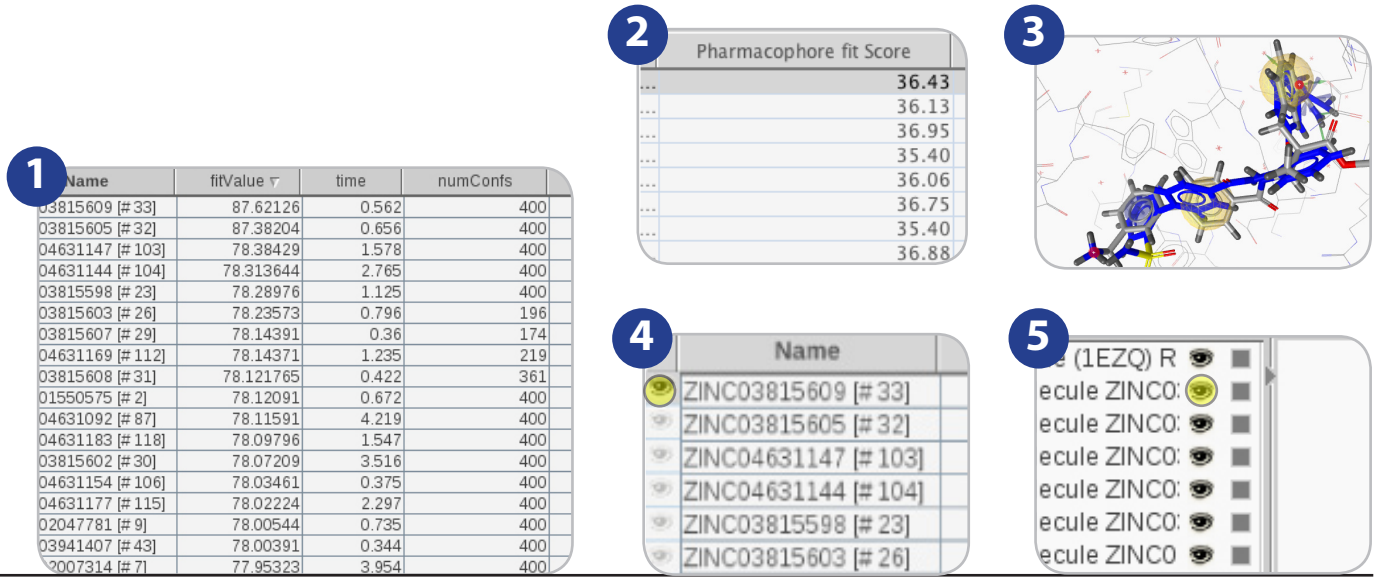
Perform virtual screening to obtain a hit list as described on tutorial card 4 'Virtual screening and comparing active/inactive hit retrieval rates' or insert external screening results from a library file via the 'File - Insert' menu entry. Results of virtual screening are depicted in the library view [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 Gaussion 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



View and rescore docking poses with pharmacophores

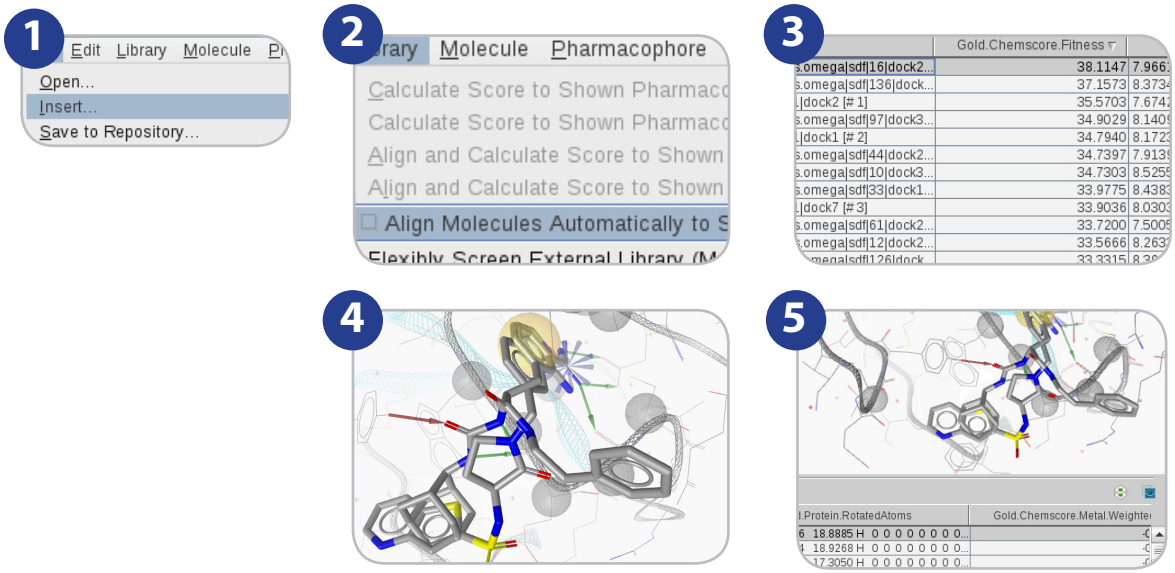
Experience level: medium
Time needed: 10 minutes
Prerequisites: docking poses from external sources

Views	Sequence	User Controls	Advanced controls (opt.)
○ Library view (spread-sheet) ○ Hierarchy view	○ 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	○ Library view (Spread-sheet) ○ 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-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



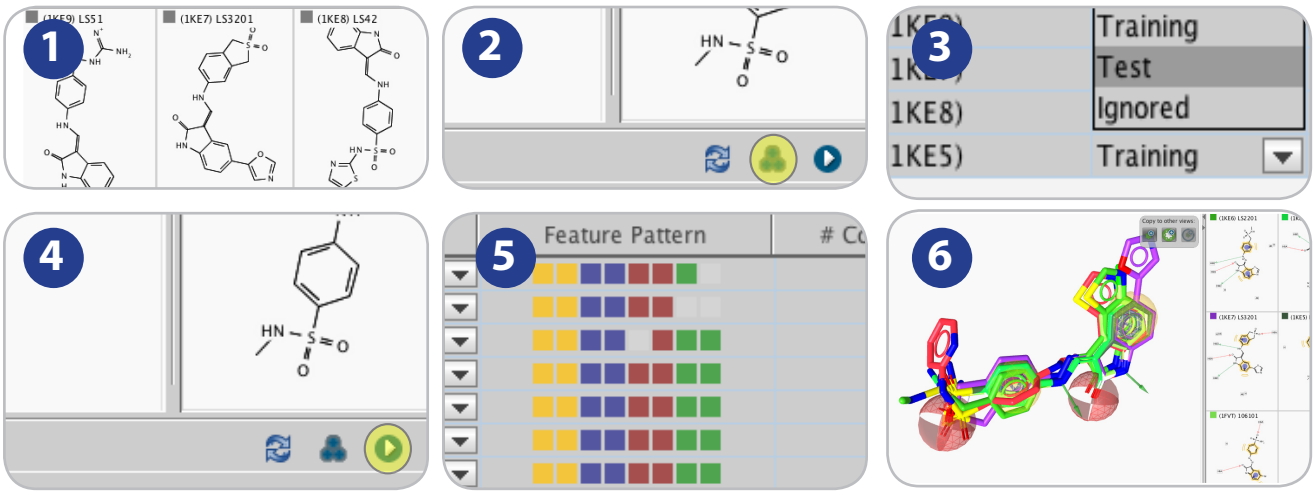
Espresso: Create ligand-based 3D pharmacophores

Experience level: intermediate
Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
○ Ligand-based Modeling	○ Import ligands ○ Cluster ligand-set in 3D ○ Generate ligand-based pharmacophore model	○ 3D Clustering ○ Generate ligand-based pharmacophore model (button or menu)	○ 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



Screen several databases using the "Screening Perspective"

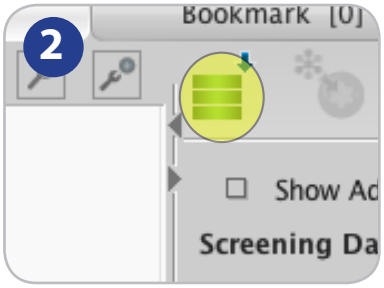
Experience level: intermediate
Time needed: 5 minutes

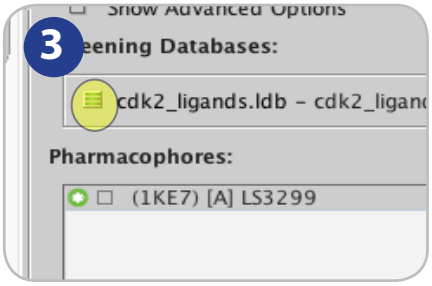
Views	Sequence	User Controls	Advanced controls (opt.)
○ Screening view	○ Add pharmacophore model to screening view ○ Load virtual database ○ Perform virtual screening	○ Load Screening Database (Button) ○ Perform Screening (Button)	○ none

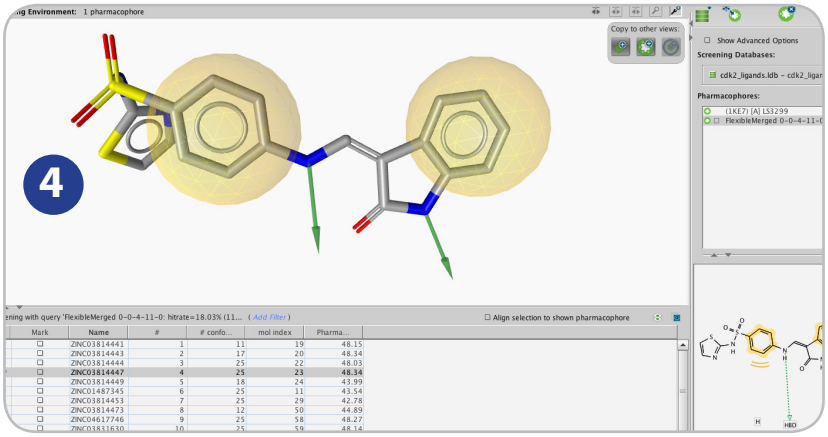
Description:
In contrast to the virtual screening procedure in the 'Structure-based Modeling' perspective which is designed for pharmacophore model refinement with respect to ligand-binding pocket properties, the 'Screening' perspective provides a fast and convenient way of performing multiple virtual 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







Advanced screening: ROC curves and model combination

Experience level: advanced
Time needed: 15 minutes

Views	Sequence	User Controls	Advanced controls (opt.)
○ Screening view	○ Add pharmacophore models to screening view ○ Load virtual database ○ Perform virtual screening ○ Plot ROC curve ○ Combine models by 'OR'	○ Load Screening Database (Button) ○ Perform Screening (Button) ○ Plot ROC Curve (Button)	○ 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 containaing actives (green database symbol), and twice on the tri-state slector 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