An Anticancer CDK2 Inhibitor Using an EHT Scheme

This tutorial introduces the MOE Pharmacophore tools in a structure-based design context. We will use an EHT pharmacophore scheme and will be working with an anticancer CDK2 inhibitor that was prepared as a bioisostere of roscovitine.

The EHT pharmacophore scheme is based on a semi-empirical approach using Extended Hueckel Theory (EHT) for generating pharmacophore annotations and features. The EHT approach takes into account ligand resonance and electron withdrawing effects and consequently the pharmacophore features generated through the EHT scheme are sensitive to non-standard interactions, such as C-H and halogen bond interactions, during pharmacophore screening. The benefits of this approach include:

- SMARTS patterns are not needed for annotations.
- Resonance effects are captured in the acceptor and donor strengths.
- Each feature has an associated strength value, so it is easy to differentiate between weak and strong features.
- Weak-weak interactions are avoided.
- New pharmacophore features through C-H and halogen bond interactions.

In this lesson, we will use an EHT pharmacophore scheme and will be working with an anticancer CDK2 inhibitor that was prepared as a bioisostere of roscovitine.

Creating a Pharmacophore Query

A pharmacophore query is created in MOE using the **Pharmacophore Query Editor**. A query consists of a set of constraints on the location and type of pharmacophoric features which can be used to search a database of molecular conformations. In this exercise, you will create a query and save it to be used later in a pharmacophore search.

Preparing the System

1. Load the structure of CDK2 inhibitor: **MOE | RHS | Open** | c:\moe2015\sample\mol\3pj8.pdb.gz. Load the structure into MOE with default settings.
2. Open the **QuickPrep** panel to fix atoms and set tethers: **MOE | RHS | QuickPrep**.
   In the **QuickPrep** panel:
   1. Set the Tether Receptor Strength to **5000**. This will keep the receptor relatively rigid.
   2. Press **OK** to accept the remaining defaults and close the panel.
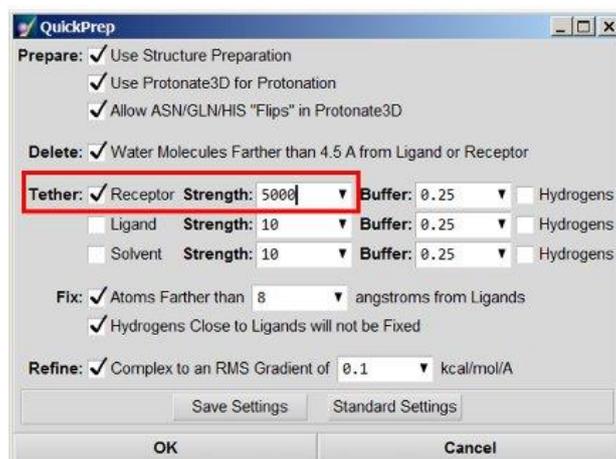
The QuickPrep calculation will begin and will prepare the complex by correcting any problems with the structure using the Structure Preparation tool. Then hydrogens are added and assigned protonation states, which is performed automatically using the Protonate3D application.

3. Display and center the active site: **MOE | RHS | SiteView**.
4. Disable the Ribbon display using the System Manager by clicking on the icon for ribbon mode and choosing the red disable icon from the ribbon style popup menu.

> **Tip:** To label the active site residues, select the receptor atoms by choosing **MOE | RHS | Select | Receptor**, and then choose **Footer | Atoms | Label: Residue**.
>
> Use **MOE | RHS | Select | Clear** to de-select the atoms.
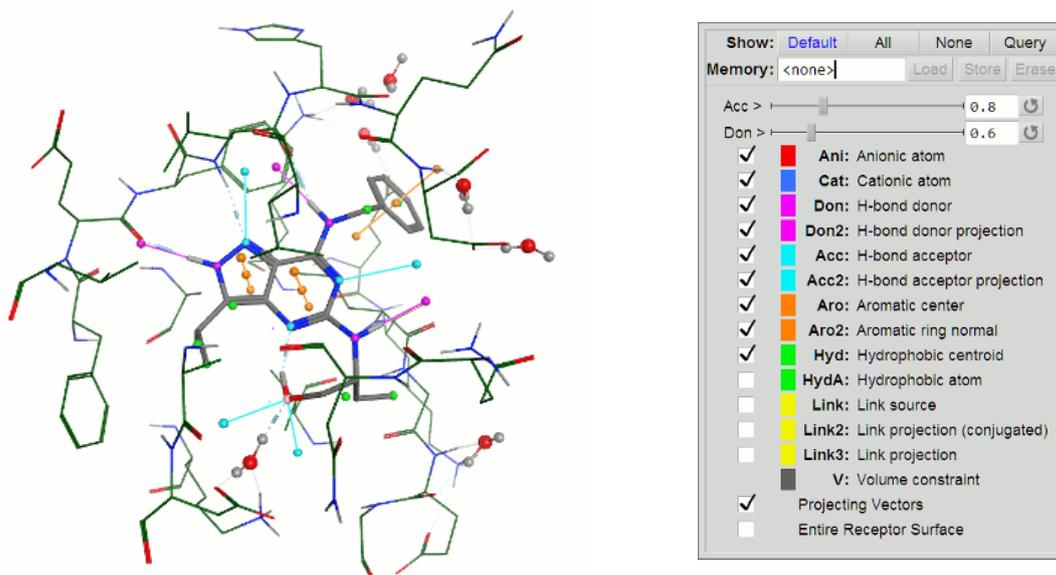
Preparing the Pharmacophore

1. Open the Pharmacophore Query Editor: **MOE | Compute | Pharmacophore | Query Editor**.

   A pharmacophore scheme defines the set of attributes that are used to construct ligand annotation points which can be matched to the query. The default scheme is called **Unified**.

2. In the **Scheme** menu, select **EHT** to use the EHT pharmacophore scheme.

   In the MOE Window, colored annotation points appear around the ligand to denote pharmacophoric regions of interest. The annotation points are color-coded by their type (green for hydrophobic, purple for hydrogen bond donor, cyan for hydrogen bond acceptor, orange for aromatic, etc.):

3. To see information on the current pharmacophore scheme, press the **Show** button to the right of the **Scheme** option menu in the Pharmacophore Editor. An information popup is displayed:
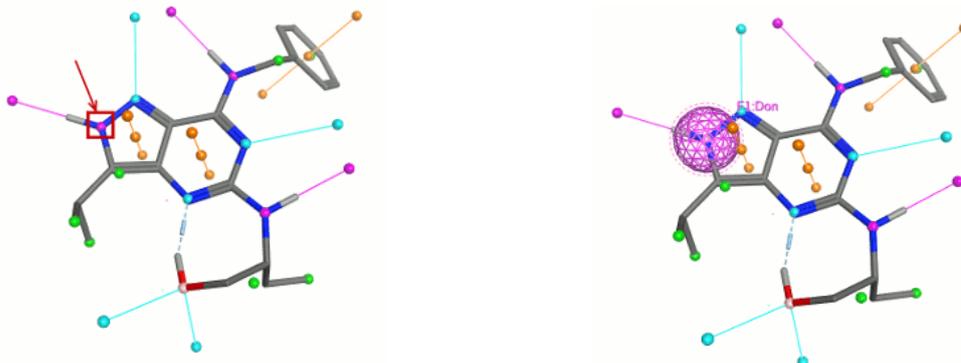

Creating a Don Feature

The first annotation feature we will create is a *Don* feature, which is a hydrogen-bond donor.

1. Click the purple *Don* annotation point on the pyrazole ring N atom.

2. In the **Pharmacophore Editor**, press **Feature**.

The new *Don* feature appears in the feature list of the **Pharmacophore Editor**, and also appears as a new feature in the MOE Window display of the ligand.

3. Modify the *Don* threshold, based on the receptor strength:
   1. Ensure the **R-Strength** option is enabled (in the top right of Pharmacophore Editor window). This shows the receptor strength in the annotation threshold equation.
   2. Open the receptor strength pull-down menu, and select **(O= in ASN, GLN, backbone)**. This specifies that the interaction acceptor atom is a backbone carbonyl, with a receptor strength of **2.0**.

Creating an Acc Feature

The second annotation feature we will create is an *Acc* feature, which is a hydrogen-bond acceptor.

1. Click the cyan *Acc* annotation point on the pyrazole ring N atom.
2. In the **Pharmacophore Editor**, press **Feature**.

The new *Acc* feature appears in the the feature list of the **Pharmacophore Editor**, and also appears as a new feature in the MOE Window display of the ligand.

We will keep the default settings for this feature, with an *Acc* threshold of **0.8** and a receptor strength of **1.25**.
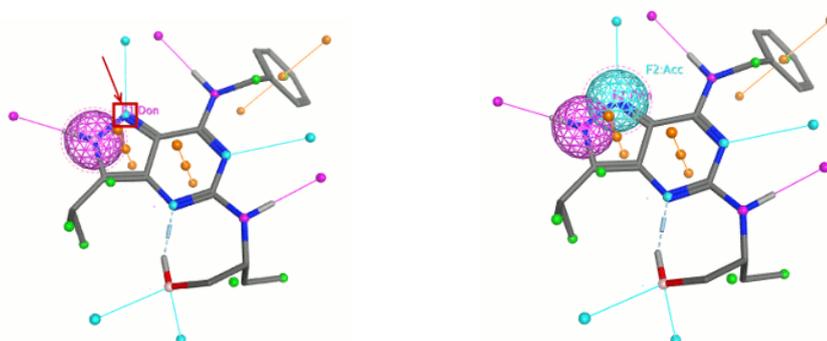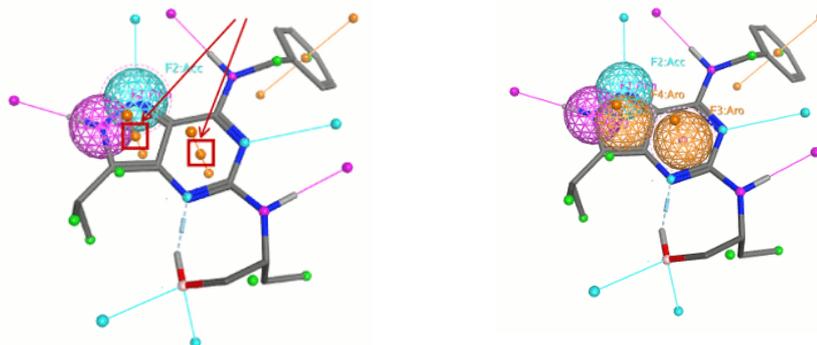

Creating Aro Features

The next annotation features we will create are *Aro* features, which are aromatic centers.

1. Click the two orange *Aro* annotation points in the centers of the aromatic rings. After clicking the first center, use Shift-Click for the second center to select it without de-selecting the first.
2. In the **Pharmacophore Editor**, press **Feature**.

The new *Aro* features appear in the feature list of the **Pharmacophore Editor**, and also appear as new features in the MOE Window display of the ligand.

We will keep the default settings for these features. The *Aro* feature has no strength settings to modify.

Creating a Don2 Feature

The final annotation feature we will create is a *Don2* feature, which is a hydrogen-bond donor projection.

1. Click the purple *Don2* annotation point projecting from the pyrazole ring NH.
2. In the **Pharmacophore Editor**, press **Feature**.

    The new *Don2* feature appears in the the feature list of the **Pharmacophore Editor**, and also appears as a new feature in the MOE Window display of the ligand. This *Don2* feature annotates the location of possible hydrogen bond partners that can interact with the *Don* feature (created earlier, which represents the N atom of the pyrazole ring).

3. Modify the *Don2* threshold, based on the receptor strength:
    o Open the receptor strength pull-down menu, and select **(O= in ASN, GLN, backbone)**. This specifies that the interaction acceptor atom is a backbone carbonyl, with a receptor strength of **2.0**.
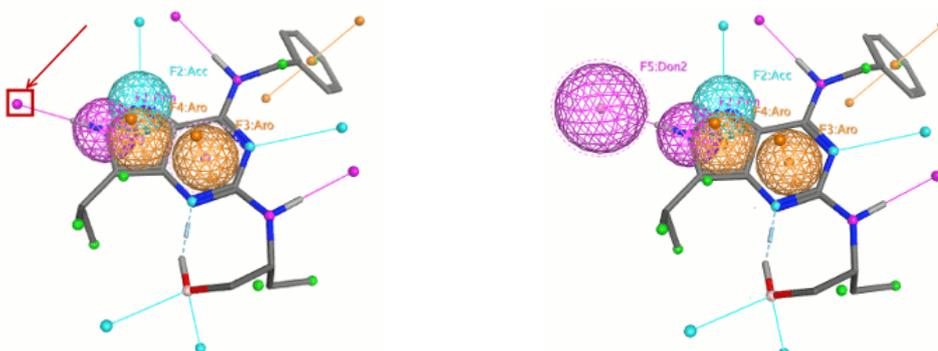
Saving a Pharmacophore Query

1. Press **Save** at the bottom of the **Pharmacophore Editor** panel to open the **Write Query File** panel.
    o In the **Write Query File** panel, type cdk2_query1.ph4 as the filename and press **OK**.

Creating Excluded Volumes around Pocket Atoms

The pocket residues can be used to create **excluded volumes** that mimic the protein pocket shape. A ligand will not match the query if any of its atom centers intersect an excluded volume.

1. Use **MOE | RHS | Select | Clear** to ensure that no other atoms remain selected in the MOE Window.
2. Use **MOE | RHS | Select | Pocket** to select the receptor pocket atoms.

3. With the pocket atoms selected, in the **Pharmacophore Editor** panel open the **Volume** drop-down menu and select **Excluded**. This will create an excluded volume sphere around each selected pocket atom.

The volume feature will appear in the feature list, and the excluded volume will be drawn in the MOE Window.

4. In the **Pharmacophore Editor** panel, change the radii of the excluded volume spheres (in the **R** textbox) from **1.6 Å** to **2.0 Å**. The default radii of 1.6 Å is typically too small to exclude all VDW clashes.
5. In the **Pharmacophore Editor** panel, press **Save**, and save this query as **cdk2_query2.ph4**.
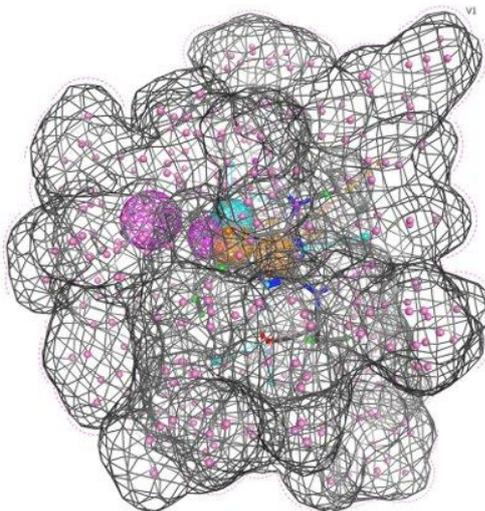
Running a Pharmacophore Search

Now that we have created a query for the CDK2 inhibitor target based on the bound structure, we will search for hits in a conformation database. The **Pharmacophore Search** panel can be launched directly from the **Pharmacophore Editor** or via **MOE|Compute|Pharmacophore|Search.**

The **Pharmacophore Search** panel is roughly divided into three sections. The top specifies the input, the middle specifies the query (and in this case it is from the Pharmacophore Editor), and the bottom section configures the output hit database. The output database by default is named **ph4out.mdb**.
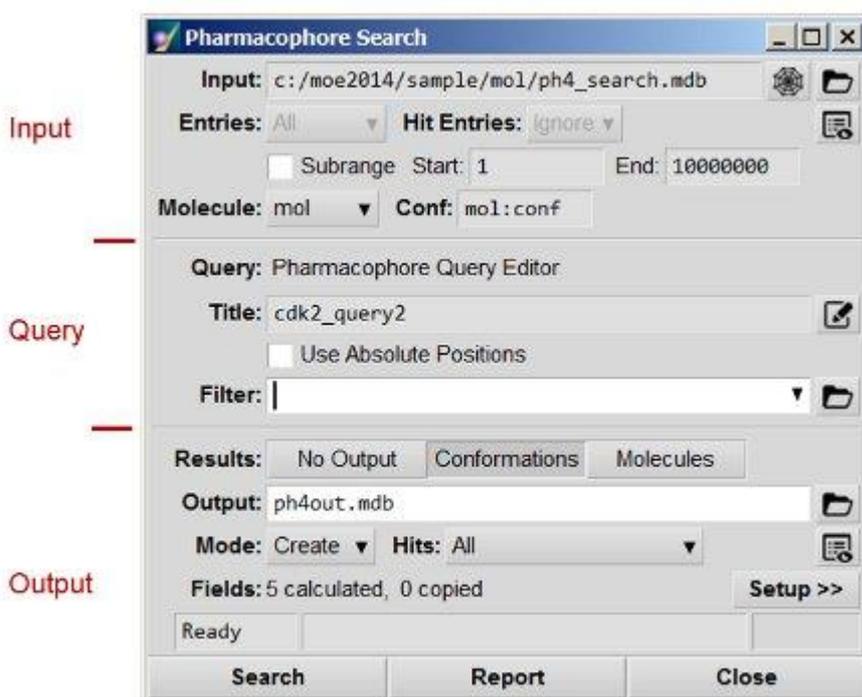
Tip: When **Hits: All** is used as indicated in the lower part of the **Pharmacophore Search** window, **all conformations** that fit the pharmacophore query will be reported – it means that from one molecule there can be many conformations as many different entries (lines in the output database). To simplify the results, choose **Hits: Best per molecule.**

1. Press the ▣ button in the Results section of the **Pharmacophore Search** panel to bring up the database of hit molecules aligned to the query, in ph4out.mdb.

Analyzing the Output

1. When the search is finished, you should see approximately 513 entries in the database. This corresponds to the total number of hits. The number of hit molecules, however, is 52 and is listed in the footer of the **Pharmacophore Search** panel. Other information is written to the **Command Line History** (Press the **SVL** button in the top right corner of the MOE Window).

   The ph4out.mdb output database is opened in the **Database Viewer**, and the highest **rscore** values are highlighted in yellow.

   The following fields are displayed in the **Database Viewer**:

   - **mol**: The hit molecules.
   - **mseq**: A unique ID for the hit molecules in the database.
   - **rmsd**: The RMSD between query features and matching ligand target points.
   - **rscore**: The sum of the individual feature rscores.
   - **rscore[F#]**: The acceptor or donor strength of matching atoms, per feature.

2. Browse the output database **DBV | File | Browse** and observe how the hit conformation fit into the pharmacophore and how well they are positioned in the context of original ligand in CDK2 receptor.
3. **Record the number of hits of the pharmacophore search.** Results of the search are summarized in **Pharmacophore Search | Report** or can be viewed in the **MOE | SVL.**

	Searched Database	Hits (output database) using less restrictive cdk2_query2.ph4	Hits (output database) using more restrictive* cdk2_query3.ph4
Molecules			
Conformations			

* Described in Additional tasks

Additional tasks

Upon consultation with teacher, add one more pharmacophore feature to create a more specific (more restrictive) query. Save as cdk2_query3.ph4 and perform the search. In the output database of this more restrictive search, select the best hits according to the **rscore** parameter.

Dock the best hits to the CDK2 receptor. In docking, the pharmacophore query (saved previously as the *.ph4 file) can be exploited in two ways:

1. **For the filtering of final poses suggested by the docking procedure.** The docking proceed as usual, the poses are generated without any knowledge of the pharmacophore. After docking, the pharmacophore filter is applied and only the poses matching the pharmacophore are reported.
2. **To guide the placement of ligand during the docking.** This can be achieved by setting the placement method to **Placement: Pharmacophore.** Pharmacophores without excluded volumes should be used (collision of ligand-receptor is prevented by the docking procedure itself).