3 Docking Ligands into Proteins

Overview

📖 NOTE

"The antithesis of rational methods for drug design is the complete reliance on chance discoveries of active ligands. High-throughput screening (HTS) is a chance-based method. The practical limitations of HTS are twofold: the volume of screen throughput that can be achieved within commercial constraints and the theoretical coverage within the diversity of the compound set being screened. If the number of compounds currently in existence is, for argument's sake, 10^8 and the number of drug-like molecules that could potentially be made is 10^{30}, the probability of a compound set of a size currently in existence showing useful statistical coverage of chemical space is minuscule."[1]

High-throughput screening methods are often best used to optimize lead molecules that are discovered in a variety of ways.[1] Rational structure-based methods represent an established approach to discovering lead compounds that in novel classes of compounds.

The screening you did in the last chapter has provided a list of potential lead compounds based upon established and known structures. Unfortunately, these structures are likely to be well-known and therefore not patentable. To create a new class of compounds that are patentable and novel, you need to create innovative structures which are also active. Designing structures based on docking novel ligands into an active site and comparing the docking to those of known active compounds is a powerful way to design new candidate drugs.

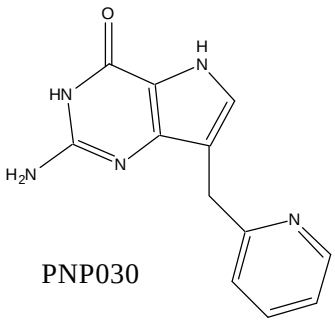
In this exercise, you will use CAChe to

- dock a ligand into a binding site by superposition
- view the docked ligand inside the active site pocket wireframe
- manually refine the position
- evaluate the quality of the binding by counting the number of binding interactions
- refine the docking with molecular mechanics

1. P. J. Gane and P. M. Dean, "Recent advances in structure-based rational drug design", *Current Opinion in Structural Biology,* **2000**, *10*, 401-404.

Background

In a previous exercise, you identified the binding site of ImmH in TB-PNP. Here, you take compound 30 (`PNP030.csf`), one of the most active inhibitors (IC_{50} 15.3 nM) of calf spleen PNP, from a class of 9-substituted-9-deazaguanine compounds and dock it into TB-PNP.[1]



PNP030

Docking by superposition

The fastest and easiest method for docking a ligand into an active site is to superimpose the ligand onto a bound ligand already in the active site and then delete the bound ligand.

To dock PNP030 into TB-PNP

Select Tool
Select Molecule
Select Similar
Select Group
Atom Tool
Rotate
Translate
Zoom

1. Choose **File | Open** and select `TB-PNP-All-Refined.csf`.

   `TB-PNP-All-Refined.csf` opens and displays the cleaned structure.
2. Choose **Edit | Group Atoms**, select ImmH from the list of **Defined Groups**, choose **G Only**.

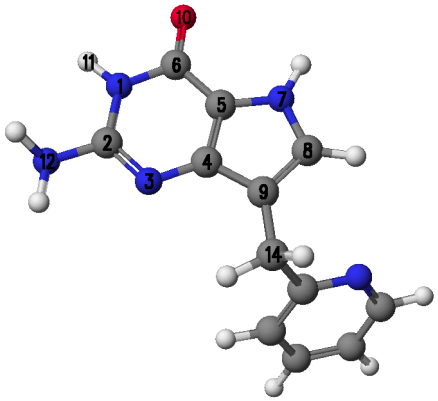
   ImmH highlights.
3. Choose **OK**.
4. Choose **View | Hide Unselected**.

   All parts of the chemical sample except ImmH disappear.
5. Choose **View | Color by Element**.

   Atoms and bonds in ImmH are colored according to element type.
6. Choose **File | Open** and select `PNP030.csf`.

1. Farutin, V., Masterson, L., Andricopulo, A.D., Cheng, J., Riley, B., Hakimi, R., Frazer, J. W., and Cordes, E. H., "Structure-Activity Relationships for a Class of Inhibitors of Purine Nucleoside Phosphorylase", J. Med. Chem., **1999**, *42*, 2422-2431.

PNP030 opens in a new 3D sample window.



7. With the Select Molecule tool and click on an atom in PNP030.

   PNP030 is highlighted.

8. Choose **Edit | Copy**.

   PNP030 is copied to the clipboard.

9. Choose **Window |...\TB-PNP-All-Refined.csf**.

   The 3D Sample Window for TB-PNP comes to the front.

10. Choose **Edit | Paste**.

    PNP030 appears in the center of the TB-PNP window.

11. Choose **Edit | Group Atoms**.

    The Group Atoms dialog appears.

12. Type PNP030 into the **Group Name** text box and choose **>> Group >>**.

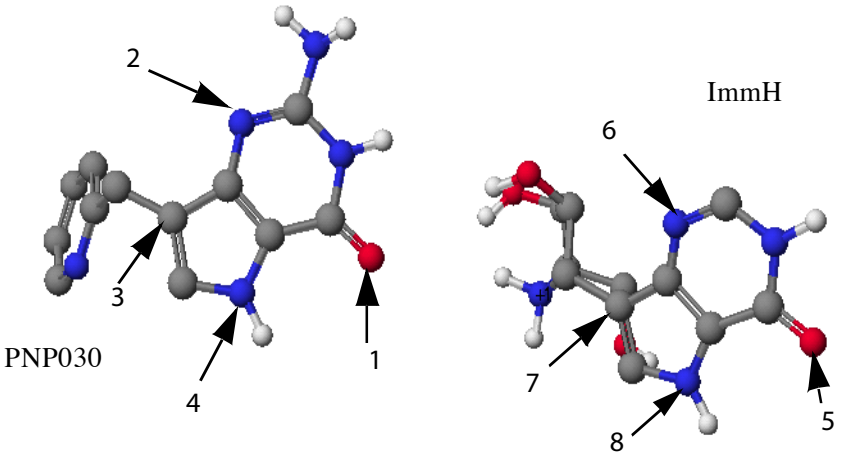
    PNP030 is added to the **Defined Groups** list.

13. Choose **OK**.

    The Group Atoms dialog closes.

14. Choose **Edit | Move Selected** and use the Rotate Tool to position PNP030 so that it is oriented the same as ImmH.

The contents of your window looks similar to this. PNP030 is on the left



and ImmH is on the right.

✍ NOTE

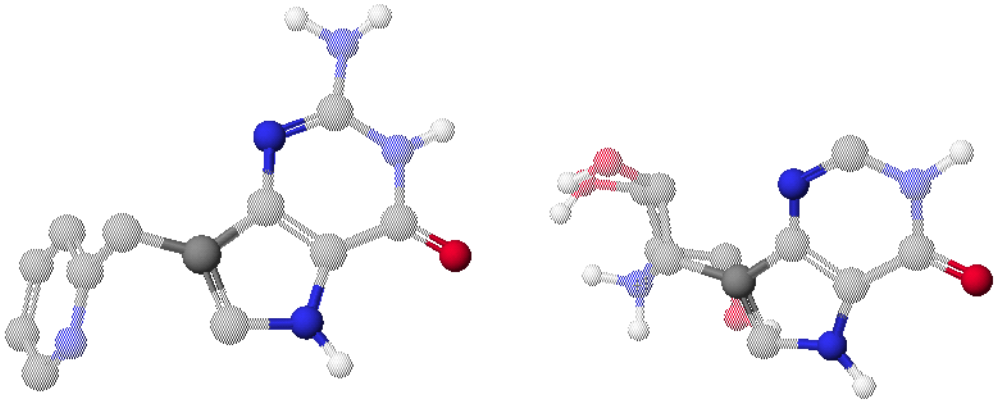
When superimposing molecules, the order of selection is important because, the first atom selected in the first molecule is superimposed on the first atom selected in the second molecule, etc.

15. With the Select Tool, click first on the carbonyl oxygen (1) in PNP030, and then hold the shift down and click on the atoms pointed to with arrows 2, 3 and 4.

    The selected atoms are highlighted. All others are dimmed.

16. With the Select Tool, hold the shift key down and - following the same order - click on the corresponding atoms in ImmH (5, then 6, then 7, then 8).

    Your structures should look similar to this



✍ TIP

The molecule containing the first selected atom is the one that moves.

17. Choose Analyze | Superimpose .

    PNP030 moves on top of ImmH so that first selected atom in PNP030 is superimposed on the first selected atom in ImmH, the second selected in PNP030 is superimposed on the second selected in ImmH, and so forth. The RMS error for the four superimposed pairs of atoms is displayed as a text label.

18. Choose **View | Color by Molecule**.

    The color of PNP030 and ImmH change.

19. Choose **View | Show Hidden**.

    TB-PNP appears with PNP030 docked in the active site and superimposed on ImmH.

20. Choose **File | Save As** and name the file `TB-PNP+PNP030.csf`.

Adjusting the docked position in the active site pocket

The protein active site is a pocket in the protein that contains the bound ligand. The surface of the protein adjacent to a bound ligand maps out the pocket. You use the adjacent surface to dock the ligand.

To view the surface of the binding site adjacent to PNP030

1. Choose **Edit | Group Atoms**, click on PNP030 in the **Defined Groups** list, and choose **G Only**.

   PNP030 highlights and the rest of the structure dims.

2. Click on ImmH in the **Defined Groups** list, and choose **S or G**.

   PNP030 and ImmH highlight and the rest of the structure is dim.

3. Choose **OK**.

4. Next choose **Analyze | Adjacent Surface - Pocket**.

   After several seconds, a transparent surface is drawn around PNP030.

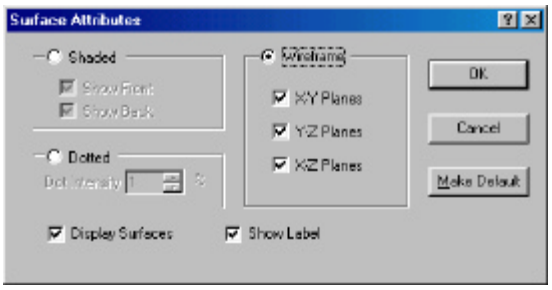
5. In the 3D Structure Window, choose the Select Tool and shift-click in the center of the "`aas 0.01`" adjacent accessible surface label.

   The adjacent accessible surface for the ImmH pocket is highlighted while PNP030 and ImmH remain selected and highlighted.

6. Choose **Analyze | Surface Attributes.**

   The Surface Attributes dialog appears.

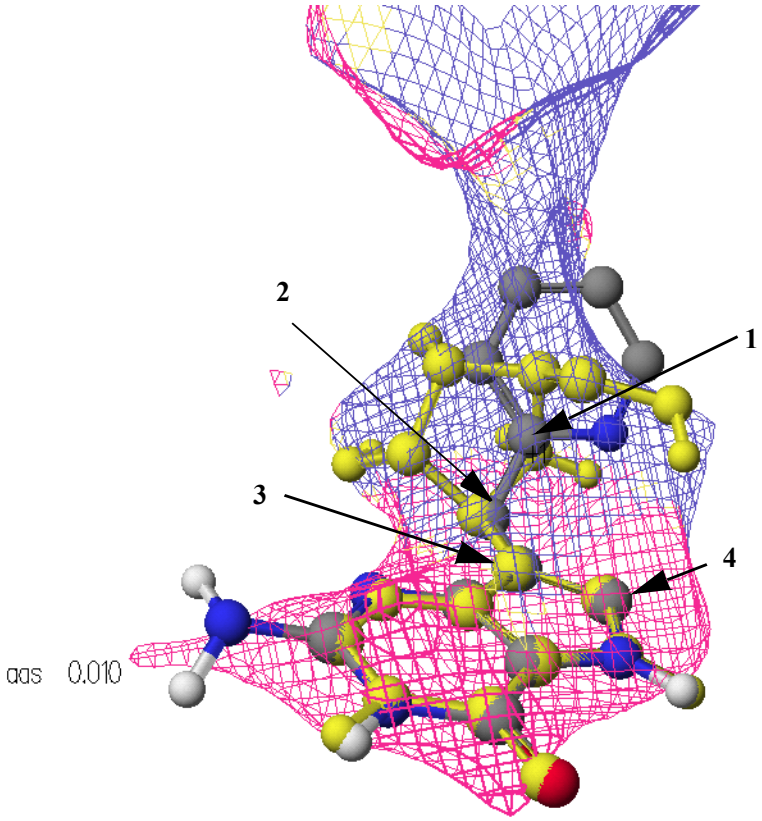
7. Choose the **Wireframe** radio button and click **OK**.



   After a few second the surface is redrawn as a wireframe.

8. Choose **View | Hide Unselected**.

You see a view similar to this.



Red marks a portion of the surface where the protein accepts H-bonds, blue marks a portion of the surface where the protein donates hydrogen bonds and cream marks the hydrophobic surface. Another way of saying this is that the red surface near carbonyl oxygen atoms in the protein while the bule portion is near the protein's hydrogen donors.

Note that PNP030's hydrogen bond donors hook through the red surface and that PNP030's hydrogen bond acceptors (C=O) should touch the surface instead of sticking through it. PNP030's hydrophobic atoms should be, but are not, buried inside the surface. This suggests that PNP030 in this conformation will not bind as well as ImmH.

9. You can set another conformation for PNP030 by rotating around flexible single bonds. With the Select Tool, click on atom **1** in the above figure. Then while holding down the shift key, click on atoms **2**, **3** and **4** in order.

   Atoms **1**, **2**, **3** and **4** highlight. The rest of the molecule dims.

10. Choose **Adjust | Dihedral Angle**.

    The Set Dihedral Angle dialog appears.

11. Type -85.0 into the Angle text box, check **Define Geometry Label** and choose **OK**.

    The pyridine ring moves to fit inside the binding pocket and the rotated

angle is labeled -85.00 degrees.

You can further refine the geometry by selecting the whole molecule and then using **Edit | Move Selected** and the Rotate Tool and Translate Tool to move PNP030 around in the wire frame pocket.

12. With the Select Molecule Tool, select ImmH and choose **Edit | Delete**.

    ImmH disappears and PNP030 is highlighted.

13. Choose **File | Save**.

    `TB-PNP+PNP030.csf` is updated.

Evaluating the docking

The binding energy depends upon the number of hydrogen bonds between the ligand and the protein and on any overlapping atoms that bump too close together.

To find the number of hydrogen bonds between PNP030 and TB-PNP

1. With the Select Molecule Tool, click on PNP030.

   PNP030 highlights and the rest of the structure dims.

2. Then choose **View | Show Hidden**.

   The rest of the structure appears. It should all be dimmed.

3. Next choose **Analyze | Label Hydrogen Bonds**.

   Distance labels appear for each hydrogen bond between the PNP030 and the protein and for each hydrogen bond internal to PNP030. You see a total of 4 intermolecular hydrogen bonds binding PNP030 and TB-PNP.

To find any bumping atoms between PNP030 and TB-PNP

1. With the Select Molecule Tool, click on PNP030.

   PNP030 highlights and the rest of the structure dims.

2. Next choose **Analyze | Label Bumps**.

   New distance labels appear for each atom pair that is too close together. Ideally, you should see no new distance labels. However, PNP030 bumps into adjacent atoms and several labels appear especially near the pyridine ring. These bumps suggest that the binding of PNP030 into a rigid receptor is not as good as that of ImmH.

NOTE

Here, PNP030 does not bump into PO_4^{3-}. This suggests the possibility that PO_4^{3-} is also bound with PNP030. If you decide that it does not co-bind, then delete PO_4^{3-} before refinement.

Refining the docking

The docking can be further refined by molecular mechanics which we will setup to allow both the receptor and the ligand to relax.

To refine the PNP030 docking in TB-PNP

1. Choose **Experiment | New**.

   The Experiment Dialog appears.

2. Choose **Property of: | chemical sample**, **Property: | optimized geometry** and **Using: | MM geometry (MM2)**.

3. Click **Start**.

   `TB-PNP+PNP030.csf` is saved, the Experiment Status Window opens and a molecular mechanics calculation using the MM2 force-field runs. When it completes (about 10 minutes), the 3D Sample Window is updated.

So what?

You have seen how the new ligand - PNP030 - could interact with TB-PNP. You have counted the hydrogen bonding interactions, noted that there is unexploited space in the active site pocket, and examined whether PNP030 bumps into other parts of the structures.

You should note that the $-NH_2$ group in PNP030 exactly fills the unexploited space in the active site discovered in the previous exercise.

This analysis has helped you understand the affinity of the ligands for TB-PNP. But a good drug is also selective. Therefore, you need to understand how ligands that bind to TB-PNP bind to related proteins such as human PNP. Selective ligands will have at least one binding interaction that is different between TB-PNP and human PNP.

In the next exercise, you will use the docked TB-NP+PNP030 structure to locate the active site in the homologous human-PNP, dock PNP030 into human PNP and look for selective interactions.