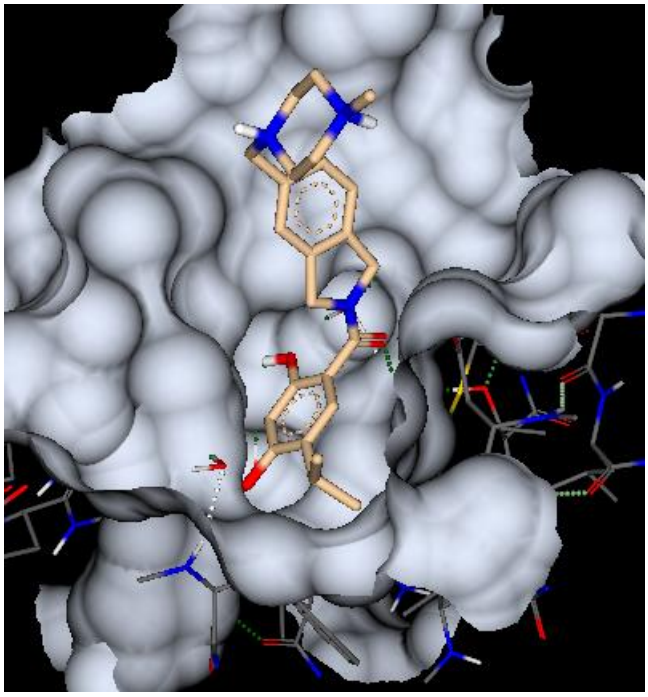
Worked Example:

Binding Affinity and Torsion Angle Analysis of Virtual Libraries

This worked example explores ways to assess the binding affinity of docked compounds. The compounds in this example were generated as part of a virtual library and are being considered for further optimisation.



The crystal structure on the right (PDB 2XJX) shows the binding site of Heat Shock Protein 90 (HSP90) with Onalespib as the co-crystallised ligand. Onalespib is a selective, potent HSP90 inhibitor that displays a long duration of anti-tumor activity. The beta resorcinol group forms a tight hydrogen bond network in the binding site, but the 5-(piperazin-1-ylmethyl)-isoindoline does not form any strong interactions with the protein.

The virtual compounds are all based on an amide coupling reaction with a beta resorcylic acid core and commercially available secondary amines.

This example demonstrates how StarDrop's SeeSAR Affinity module can be used to consider binding affinity and torsion angle analysis for 3D poses generated by any docking software. If you have just completed the SeeSAR Pose worked example, then you can carry on directly without reloading the initial file.

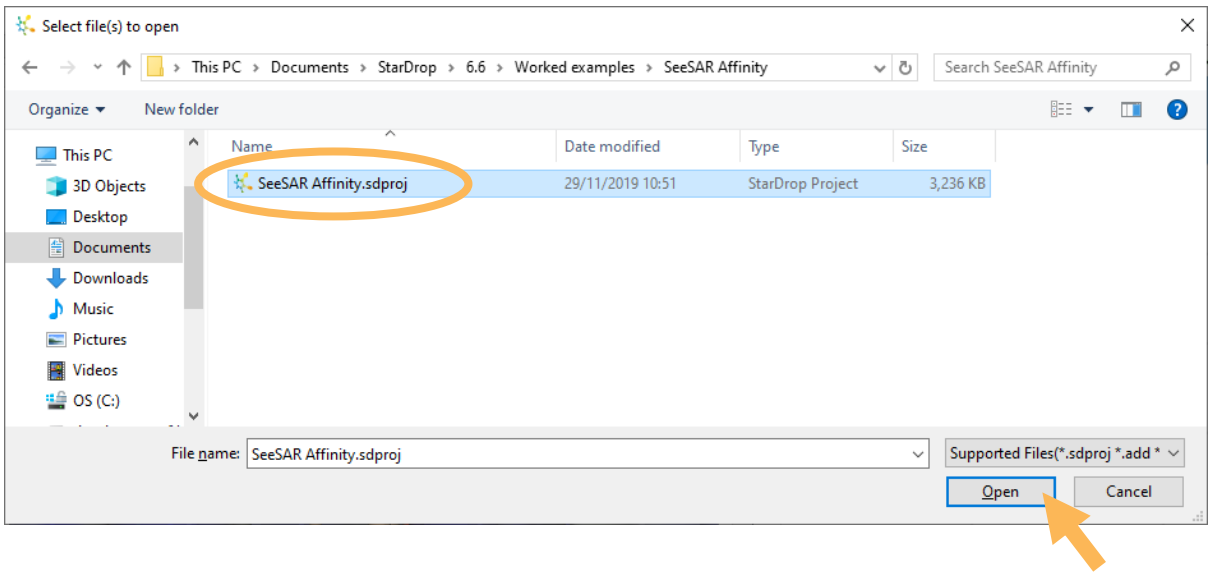
optibrium

Step-by-step instructions for all the features you will need to use in StarDrop are provided, along with screenshots and examples of the output you are likely to generate. If you have any questions, please feel free to contact stardrop-support@optibrium.com.

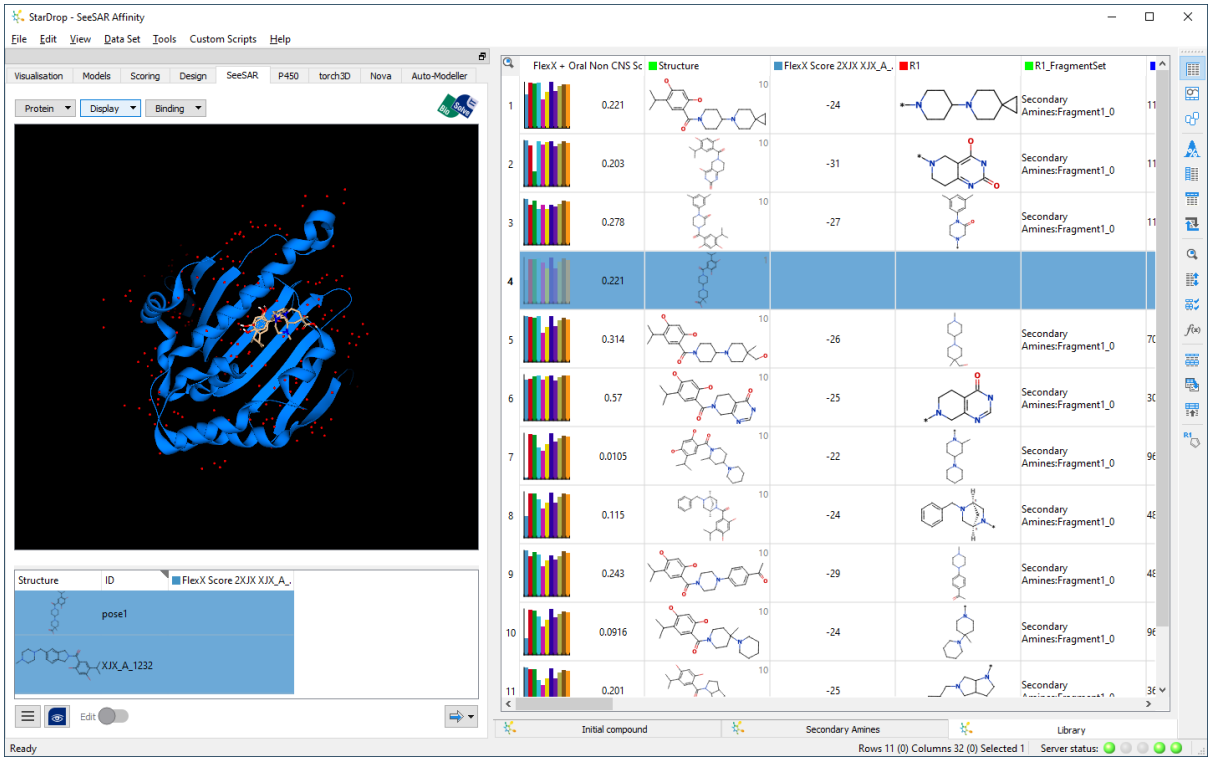
Exercise

If you have just completed the worked example “3D Analysis of Virtual Libraries”, then you already have the data set open with the selected compound displayed in its binding site and you can skip to page 4.

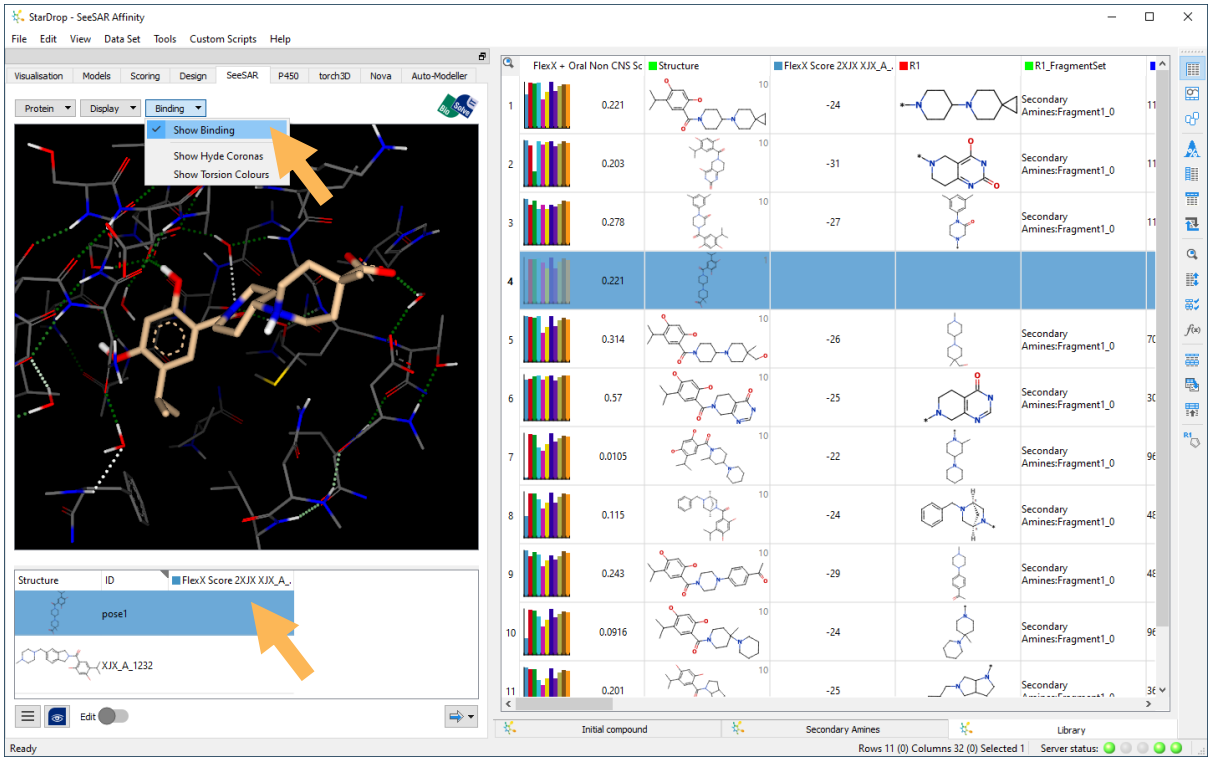
- In StarDrop, open the file **SeeSAR Affinity.sdproj** by selecting **Open** from the **File menu**.



On the left, in the SeeSAR area, the protein HSP90 is displayed with its secondary structure and the co-crystallised ligand, Onalespib. The data set on the right contains 11 compounds from the library, the first of which is also selected and displayed in the 3D viewer.

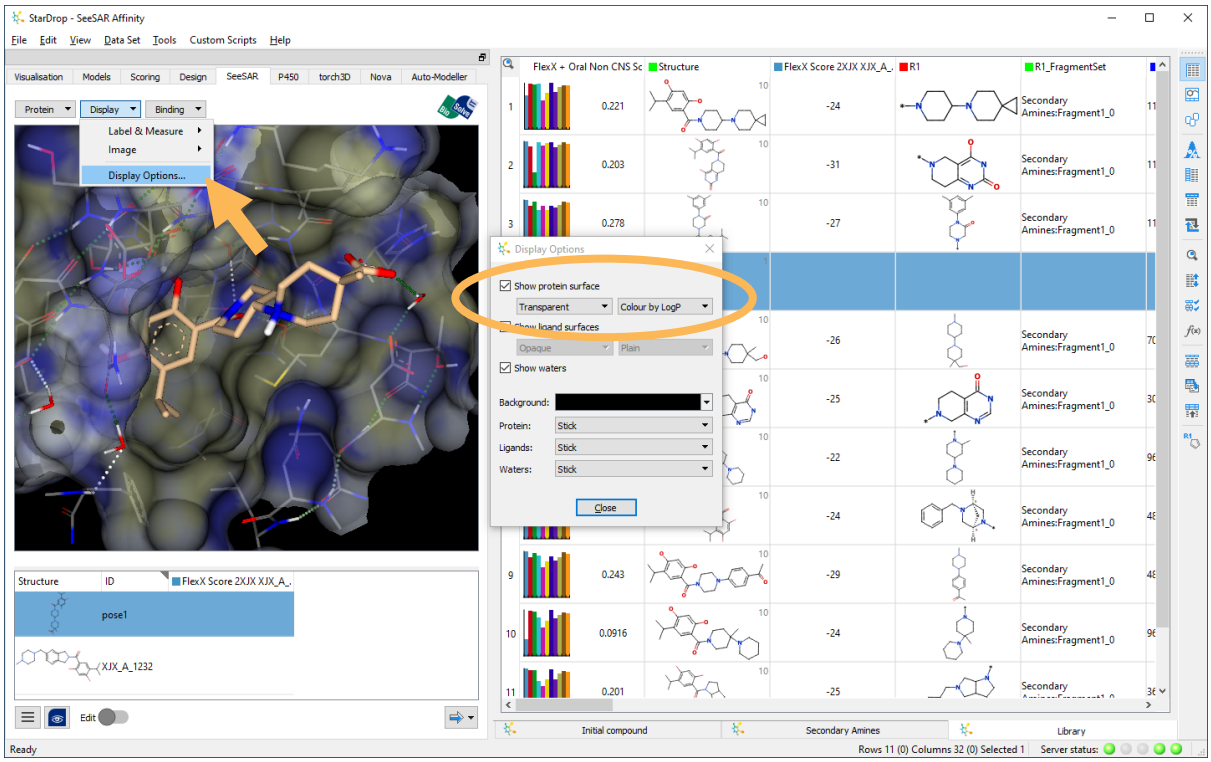


- From the table below the 3D viewer, select the first row to display this ligand without the co-crystallised structure.



- From the **Binding menu**, select **Show Binding** to focus on the binding site.
- From the **Display menu**, choose **Display Options**.

- In the **Display Options dialogue**, tick the **Show protein surface option** and choose **Transparent** and **Colour by LogP** from the menus.



- Close the **Display Option dialogue**.

This data set already contains a column showing docking scores; however, these values don't necessarily provide a good indication as to the binding affinity we might expect. The SeeSAR Affinity module enables us to use BioSolve IT's HYDE scoring that provides a consistent approach for describing hydrogen bonding, the hydrophobic effect and desolvation. It relies on HYdration and DEsolvation terms which are calibrated using the octanol/water partition coefficients of small molecules. The calibration does not employ measured affinity data and therefore HYDE is generally applicable to all protein targets. HYDE reflects the Gibbs free energy of binding while only considering the essential interactions of protein–ligand complexes.

With this in mind, during our analysis we will consider differences between the estimated binding affinities for the compounds in this library and their docking scores.

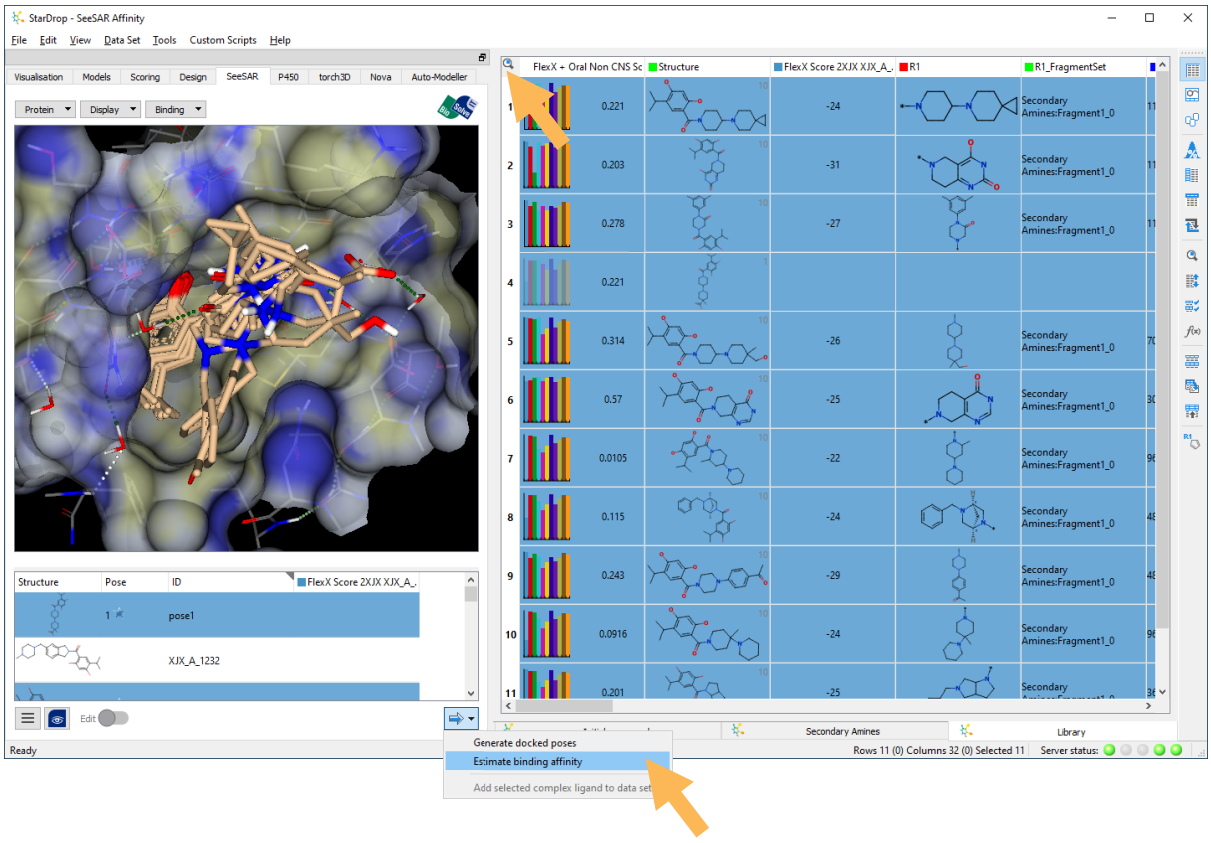
- Select the whole data set by clicking the button in the top-left corner of the data set table (just below the search icon).

- From the  menu at the bottom of the SeeSAR area, choose **Estimate binding affinity**.

  Note: This will generate poses for the entire data set. To generate poses for individual compounds, select these rows in the data set first.

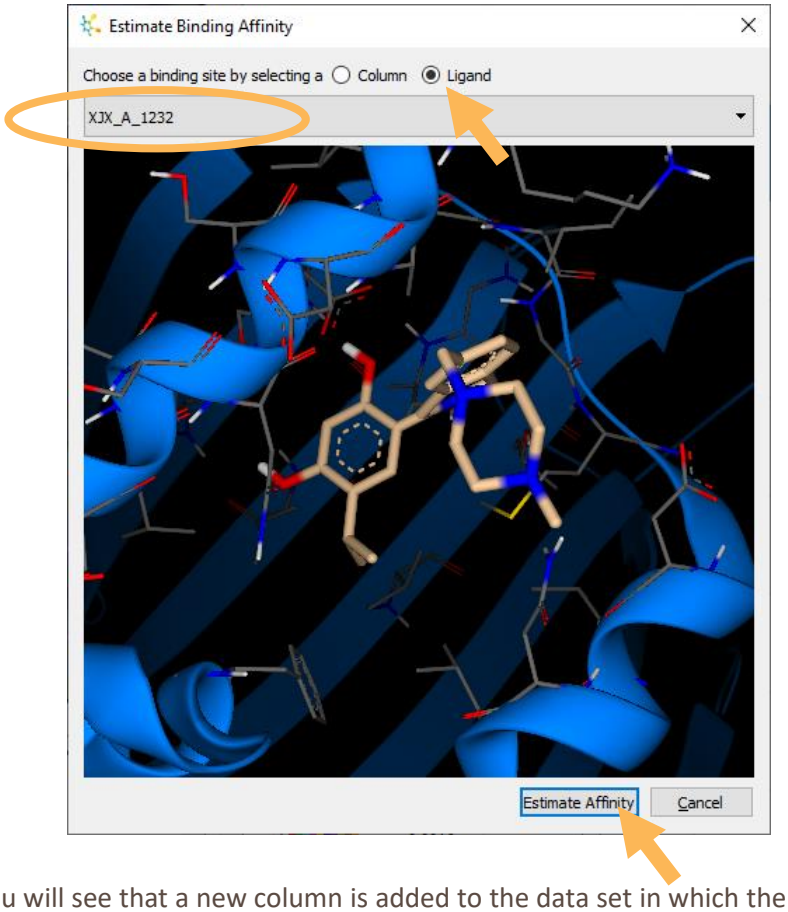
You will see a warning telling you that you are about to generate estimated binding affinities for all the poses in the data set.

- Click **OK**.



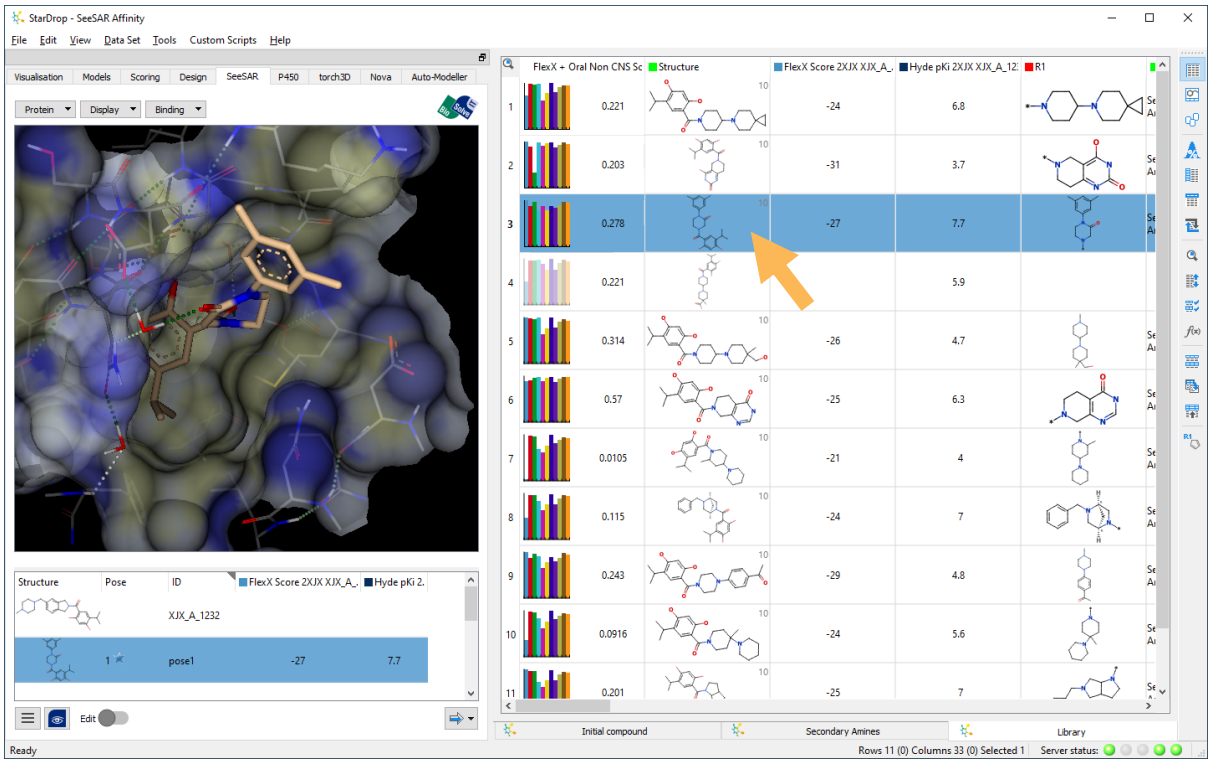
When estimating binding affinities, we need to define a binding site. A binding site can be defined either by selecting a column of docking results (for which a binding site has already been specified) or by choosing an individual ligand. In this case we will use a ligand, specifically the co-crystallised ligand, Onalespib.

- Select the **Ligand** option and choose **XJX_A_1232** from the list.
- Click the **Estimate Affinity button** to start the process.



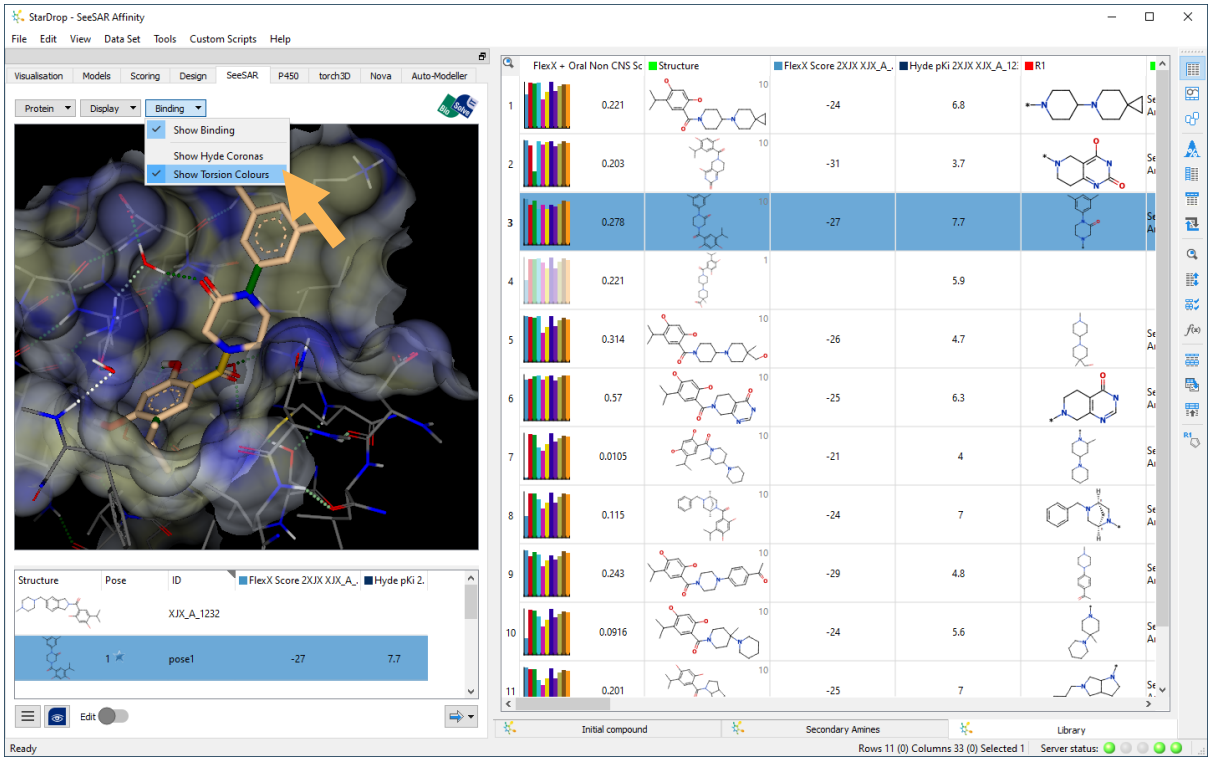
You will see that a new column is added to the data set in which the binding affinity (HYDE score) for the compound will be displayed once the calculation has completed.

- When all the calculations have completed, select the compound in **row 3**.

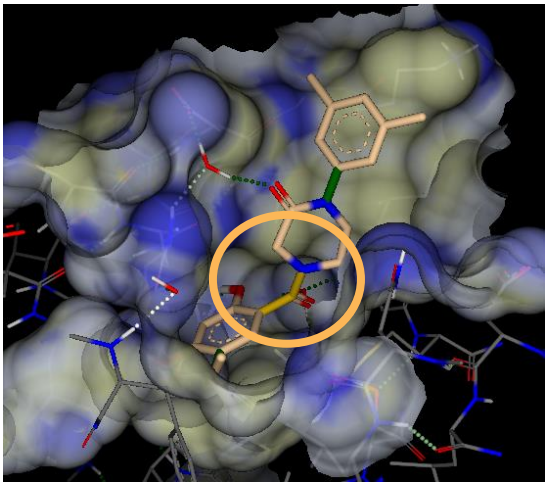


Selecting any row in the data set enables you to view that compound and its poses in the 3D viewer. Using the mouse you can rotate, zoom and pan the view to look at the compound in the binding site. The selected compound has an estimated binding affinity (pK_i) of 7.7 but one of the properties we may wish to analyse when exploring our docking results is torsion angles.

- From the **Binding menu** select **Show Torsion Colours**



This analysis is based upon a comparison with the Torsion Library, developed jointly by F. Hoffmann-La Roche and University of Hamburg that contains hundreds of rules for small molecule conformations which have been derived from the Cambridge Structural Database (CSD). Those that lie within the first tolerance interval (i.e. those seen frequently) are coloured green. Those angles observed that lie outside the first tolerance interval (i.e. those seen less frequently) are coloured yellow. Any bonds coloured red reflect highly unusual torsion angles, which are therefore questionable.



We can see that the two bonds around the amide in the selected compound are coloured yellow and so this suggests that this conformation might be strained. This is something we should be aware of, because if this is the case then it is likely to have an impact upon the binding affinity.

- From the **Binding menu** deselect **Show Torsion Colours**

It is important to also note that for some compounds we can see that, despite having a good docking score, the estimated binding affinity is very low (e.g. row 2 in the data set which has the best docking score).

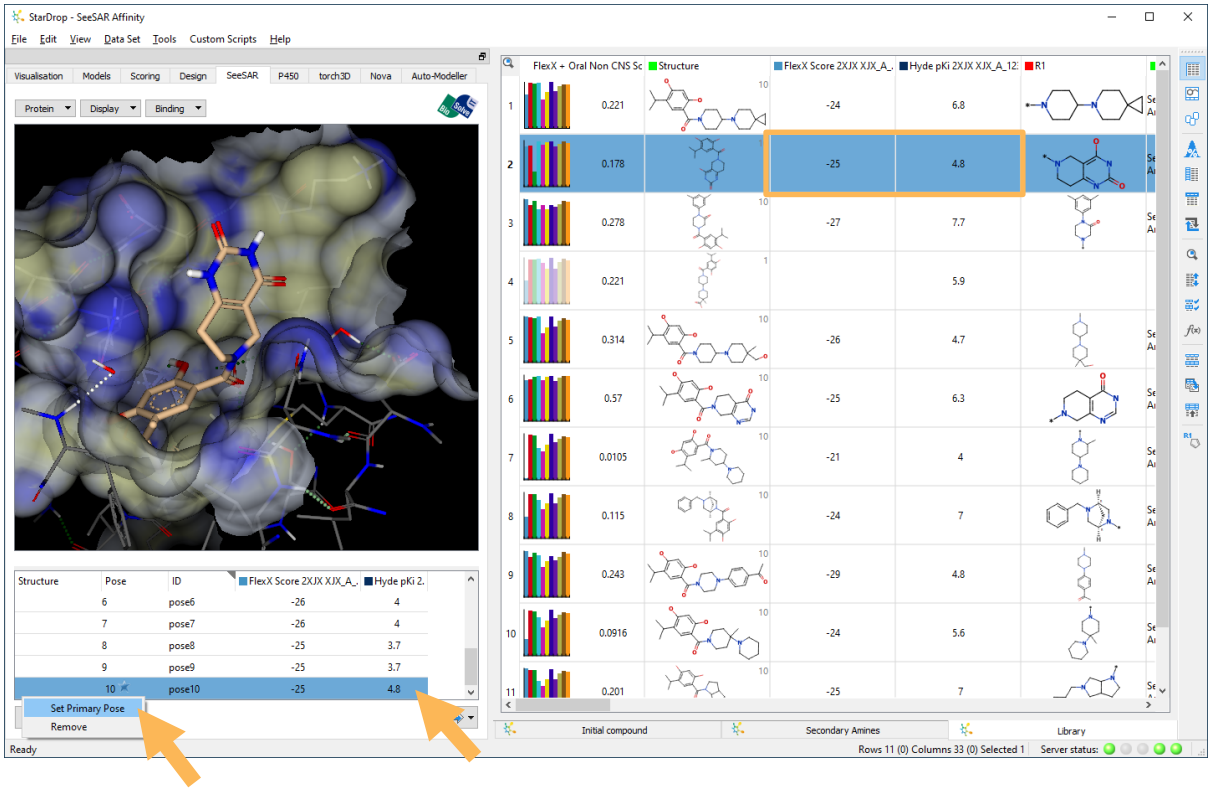
- Select the compound in **row 2**.

For this compound we can see that the pose represents an alternative tautomer (compared with the original 2D structure) which has been generated to optimise the hydrogen bonding with the protein.

Some of the other poses for this compound have better estimated binding affinities (although they are all still quite low). The individual poses are listed in the table below the 3D viewer and alongside each is its docking score and estimated binding affinity.

- Select **pose 10**.

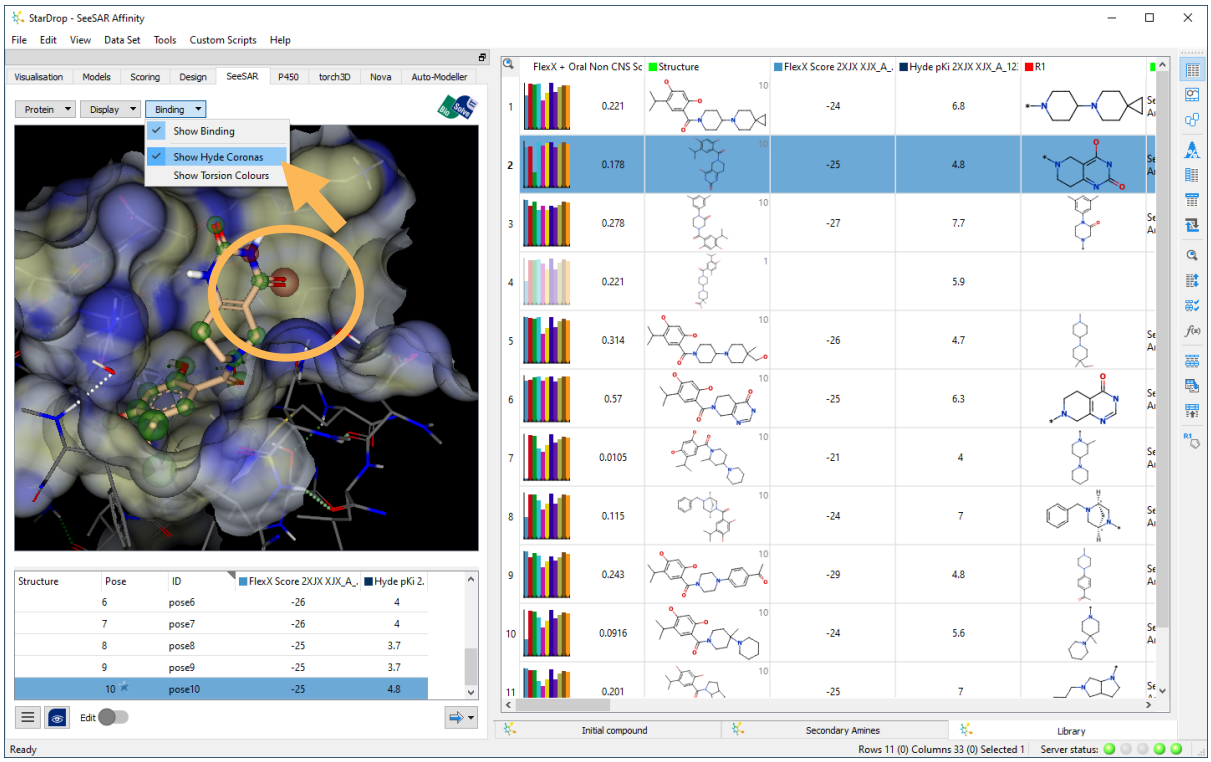
This pose does not have such a good docking score but has a better estimated binding affinity (4.8). If you scroll back to pose 1 you will see that it has a ★ against it to indicate that this is the primary pose (i.e. the values for this pose will be displayed in the StarDrop data set). We can change the primary pose to be pose 10 based upon its better estimated binding affinity.



- Right-click on pose10 in the table and from the menu choose **Set Primary Pose**.

To understand better why there is a discrepancy between the docking scores and estimated affinities we can use HYDE coronas to provide an atom-based overview of the affinity.

- From the **Binding menu** select **Show Hyde Coronas**.



The coronas enable direct visualisation of the HYDE score where:

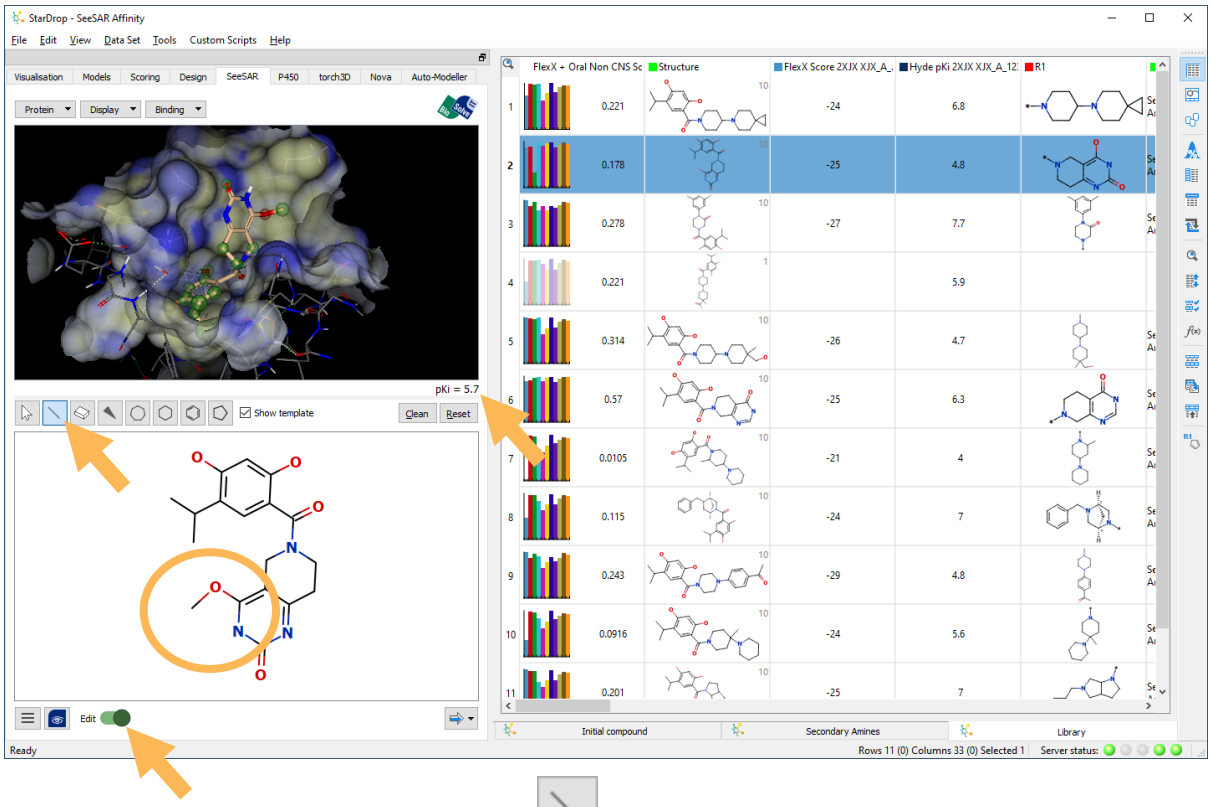
- A green corona indicates that the atom is making a favourable overall contribution to the HYDE score (estimated binding affinity)
- A red corona indicates an unfavourable contribution
- The magnitude of the corona sphere is proportional to the contribution to the score
- The absence of a corona indicates that the atom is not estimated to have a significant impact on the binding affinity

For this ligand, most of the coronas are green, but a large red corona around the carbonyl particularly stands out. This is because the carbonyl would form a strong hydrogen bond acceptor in solution, but here in this bound conformation it represents an unsatisfied hydrogen bond. As such, this has a negative impact upon the binding affinity of this conformation.

We can perform this same analysis for any conformations loaded into StarDrop as a data set.

At this stage, the next steps we might try would be to modify the carbonyl to reduce its negative influence on the binding affinity. The last steps described below are optional, if you have the SeeSAR Pose module available; however, if you have configured the Pose Generation Interface you could try the same modifications using your own docking software.

- Click on the **Edit switch** at the bottom of the **SeeSAR area** to display the 2D editor.



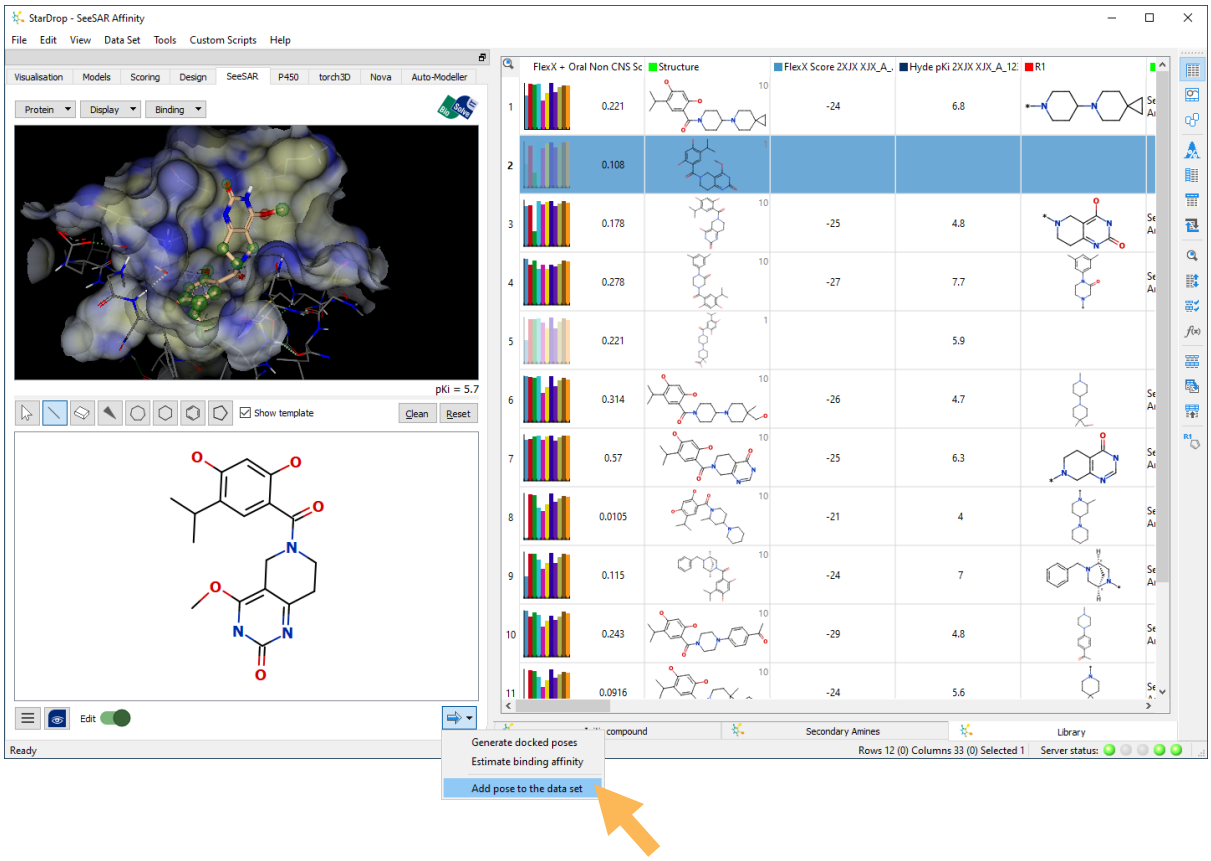
- In the sketch area, use the **Bond tool**  to form the enolic tautomer of the pyrimidinedione ring and add a methyl group to form the methoxy derivative, as shown above.

Hint: To specify an element, hover over an atom and type the element symbol, in this case “O”. Bond types (single, double, triple) may be cycled by clicking on a specific bond.

As you edit the compound, you will see a message saying “Generating pose” below the 3D viewer and a new pose will be displayed within a few seconds.

We can see from the coronas that the oxygen of the methoxy is still having a small negative impact, but overall this group appears to be less problematic than the carbonyl. This is reflected in the estimated affinity which has now increased by a log unit to 5.7. To keep this idea for further consideration we can add it to our data set.

- From the  menu at the bottom of the SeeSAR area, choose **Add pose to data set**.



If you are using alternative docking software, then this library can also be evaluated by docking in the HSP90 binding site using StarDrop's Pose Generation Interface to provide seamless integration with docking models from third-party platforms. Whilst this is beyond the scope of this exercise, if you would like to learn more about the Pose Generation Interface, please visit the following link in our online community videos:

https://www.optibrium.com/community/videos/introduction-to-stardrop-modules-and-features/375-pgi