PDBe & PDBe-KB ligand view tutorial

This tutorial aims to give you some hands-on experience of finding protein structures on the PDBe website and viewing them in 3D.

NAVIGATION – part I

Go to PDBe.org

(red text in the tutorial means you need to do something)

- You should see the following header bar:

- Your search begins with the search bar at the top right.
  Type "**Asundexian**" into the search bar.
  Publication outlining the drug discovery process for this clinical trial drug candidate: https://europepmc.org/article/MED/37669040
- Typing generates autocomplete results, grouped into different fields. The number next to each option tells you how many PDB entries are associated with it. In this case, there is only one result, and it corresponds to the number 1 – one search result / experimentally-determined structure. Click on the option.
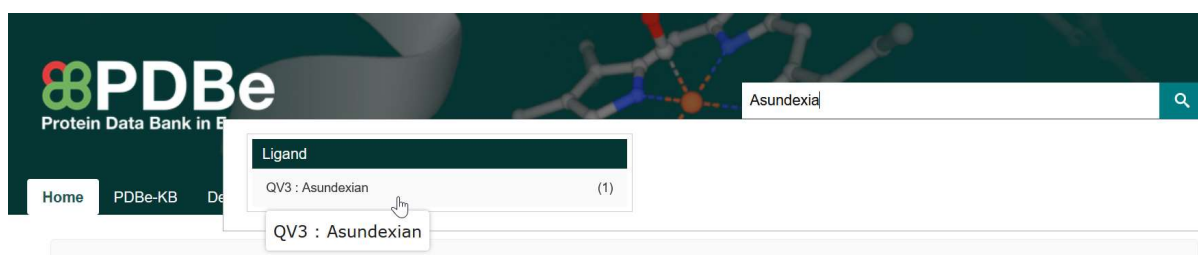
- The first view has several things you can explore for each 'hit', highlighted in the next image:
  1. View the PDB entry page for that structure.
  2. View the structure in Mol*, our 3D visualisation software.
  3. Download files related to the entry, e.g. archive files, validation report, experimental data etc.

- An alternative way to get to the entry:
  - Go to entry 8bo3 by typing "PDBe.org/8bo3" into your browser.

- This takes you to the entry page for a specific Coagulation factor structure.
  - You should see the page displayed below:

- The first thing we will do is view the structure in 3D using Mol*
  - click '3D Visualisation' highlighted in the red box above

NAVIGATION – part II

Viewing the structure in 3D

- There are a number of options on the top of the viewer, including clicking 'Everything' so both macromolecules and ligands can be scrolled through, and the aspect of interest be selected (*i.e.* QV3).
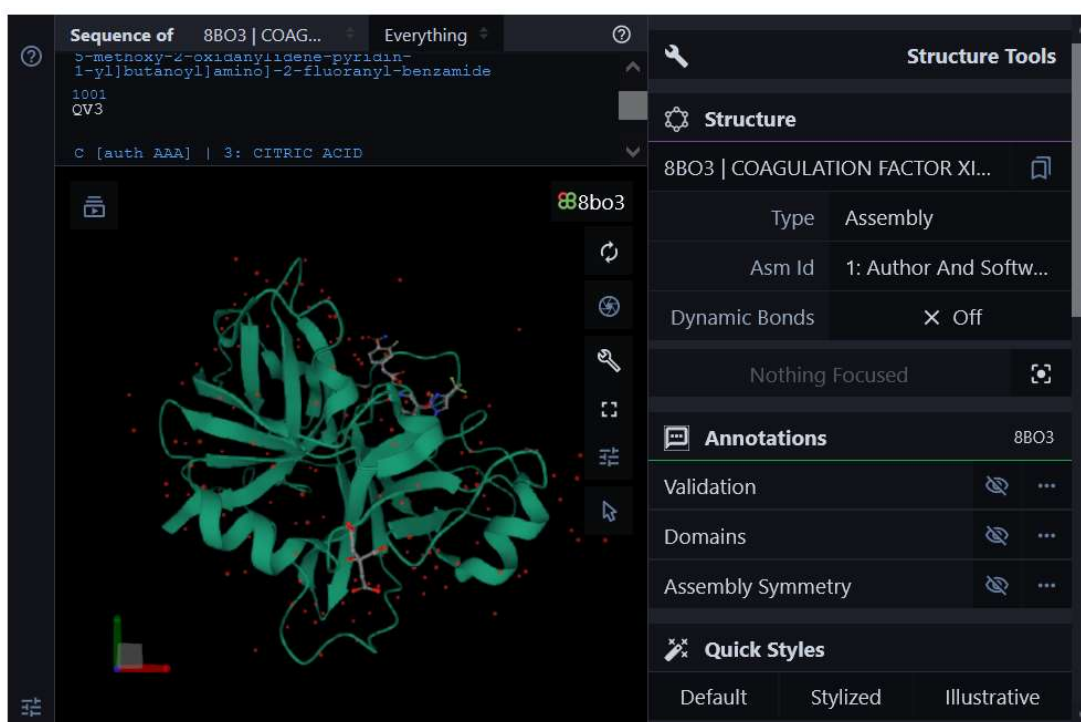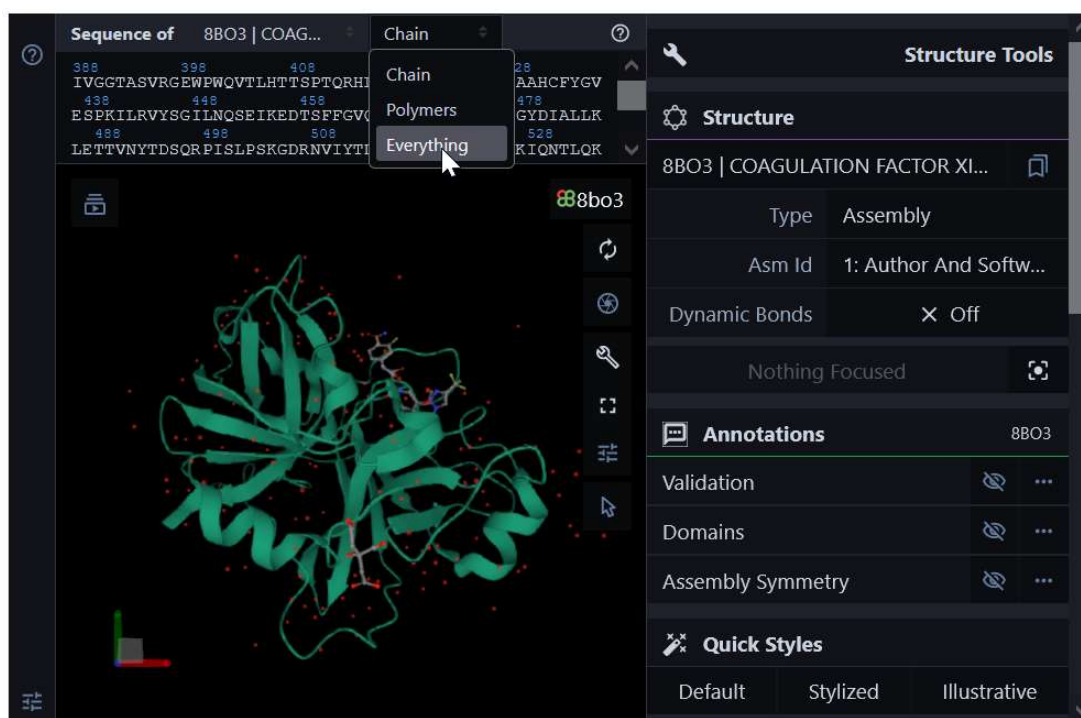
- There are also number of options on the right-hand side of the viewer to change how you visualise the structure.
- One can turn "on" and "off" the view of these components by clicking on the eye icon (highlighted in the image above)
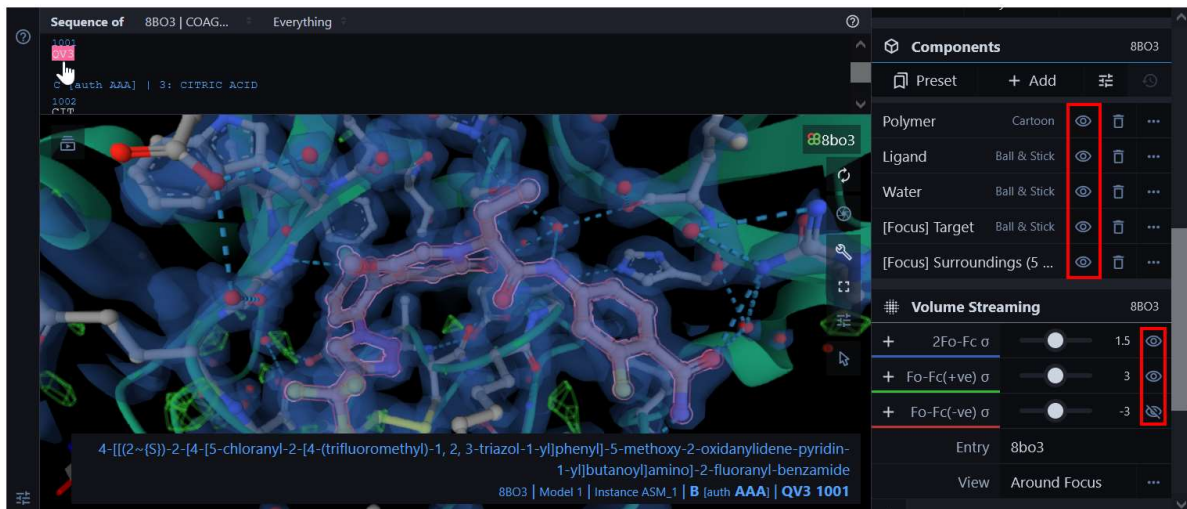- There are different parts of the structure:
  - **<u>Polymer</u>** – this includes any polymeric molecules, such as proteins and DNA.

    Question 1:
    What the 'type' of representation of the protein listed in the Mol* interface set to by default? What does this representation highlight?

  - **<u>Ligand</u>** – This includes any other molecules which are not part of a polymer (inhibitors, co-factors, substrates, ions etc). Usually these are much smaller than the polymers. We colour these by element by default

    Question 2:
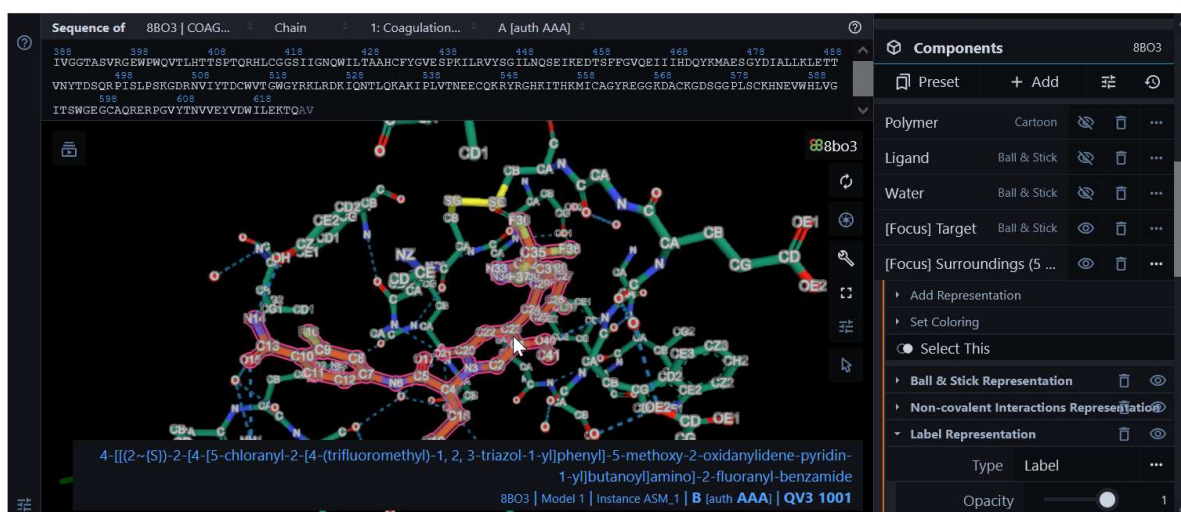   Can you work out which elements are involved in the inhibitor in this structure?
    (Hint: we usually don't show hydrogens on the molecule)

  - **<u>Water</u>** – Most PDB structures contain water molecules as proteins prefer to be hydrated. Therefore, individual water molecules are also found in the structure.

 - **<u>[Focus] Target</u>** and **<u>[Focus] surrounding (5A)</u>** – this only appears upon clicking on a ligand.

OPTIONAL:

- The right-hand side of the viewer has multiple options for viewing things in different ways. Have a go playing with these options and try to do the following tasks.

  - *Turn various parts of the structure so focus is on ligand binding site.* Click on the eye icons to turn off under '**Volume Streaming**' and '**Polymer**'. Click on the eye icon to turn off '**Ligand**' and '**Water**'.

  - *Change contrast between ligand and surrounding.* Click on the 3 dots next to '**[Focus] Surroundings**'. Click on the existing '**Ball & Stick Representation**' and it will expand revealing multiple options. Click on the 3 dots next to the '**Colour Theme**' and click on the '**Element Symbol**' adjacent to the '**Carbon Colour**'. The '**Element Symbol**' should be bold, but if you click on '**Entity id**', it should become bold.

  - *Add atom labels.* Click on the 3 dots next to '**[Focus] Surroundings**'. Click on the existing **'Add Representation'** and it will expand revealing multiple options. Click on '**Label**' and '**Label Representation**' will appear. Click on the existing '**Label Representation**' and it will expand revealing multiple options. Click on the 3 dots next to '**Label** and click on the '**Residue**' adjacent to the '**Level**' and change to '**Element**'.

From the publication:

oral FXIa inhibitor
asundexian (BAY 2433334)
clinical candidate
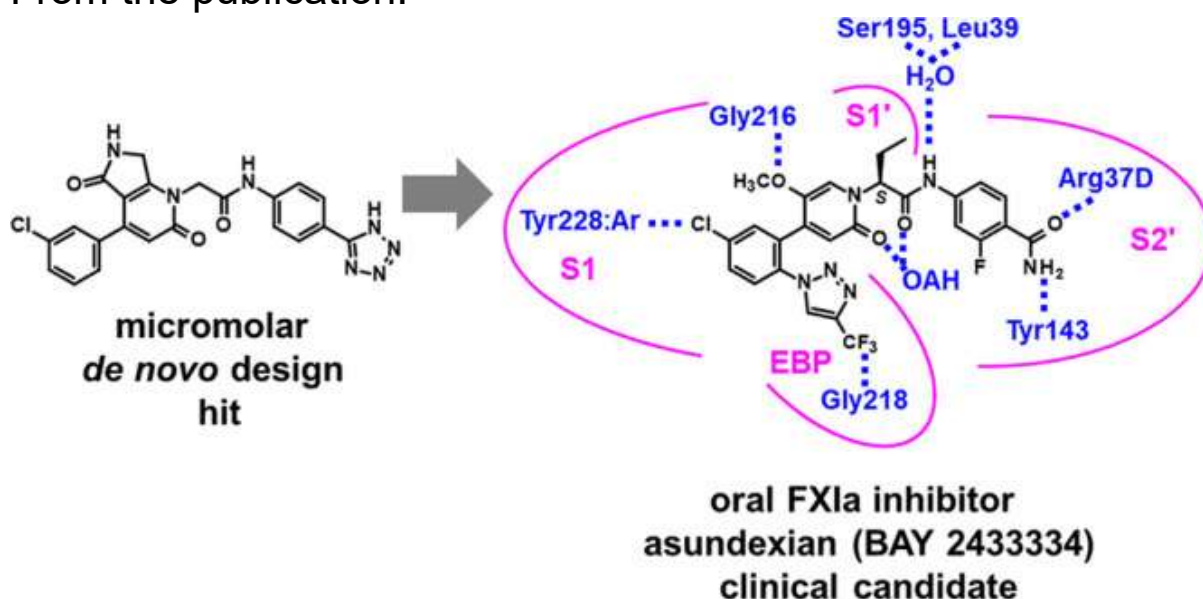
Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders

Susanne Roehrig, Jens Ackerstaff, Eloísa Jiménez Núñez, Henrik Teller, Pascal Ellerbrock, Katharina Meier, Stefan Heitmeier, Adrian Tersteegen, Jan Stampfuss, Dieter Lang, Karl-Heinz Schlemmer, Martina Schaefer, Kersten M. Gericke, Tom Kinzel, Daniel Meibom, Martina Schmidt, Christoph Gerdes, Markus Follmann, and Alexander Hillisch

Bonus Question 3:
Can you work out which atom id in the **Asundexian** has a water-coordinate-bond to two amino acids in the protein target?

Ligand binding sites – part I

Viewing small molecules bound to proteins

- Return to the entry page for 8bo3
  - Either close the Mol* viewer or go to PDBe.org/8bo3
- The small molecules in the PDB entry are displayed in the 'Ligands and Environments' section (in the red box below).

- Next, we will go the ligands page for that specific small molecule
  - Click on the image of the small molecule or three letter code
- The grey banner at the top of the page gives you some information about the molecule.
  - This includes the chemical formula of the molecule and its molecular weight in Daltons.
  - Question 4:
    What is the molecular mass of the **Asundexian**?
  - There are also two interactive images of the small molecule showing its interactions with the protein in a 3-dimensional environment view.

- The 'Environment details' section has two views:
    - On the left is an image displaying the small molecule and all of the amino acids that form the binding site for this molecule in a 2D depiction.
    - On the right is the Mol* viewer, displaying the same data in 3D. The experimental data (electron density map, since this X-ray crystallography structure) is also shown.

- The image on the left displays the bound small molecule. The amino acids in the binding site are shown in different ways:
    - There is a colour scheme highlights the different amino acids and helps in navigating the type of interactions between the ligand and the protein.
- Can you navigate to the view on the next page?
  (Hint: which icon opens the 'Menu'?)
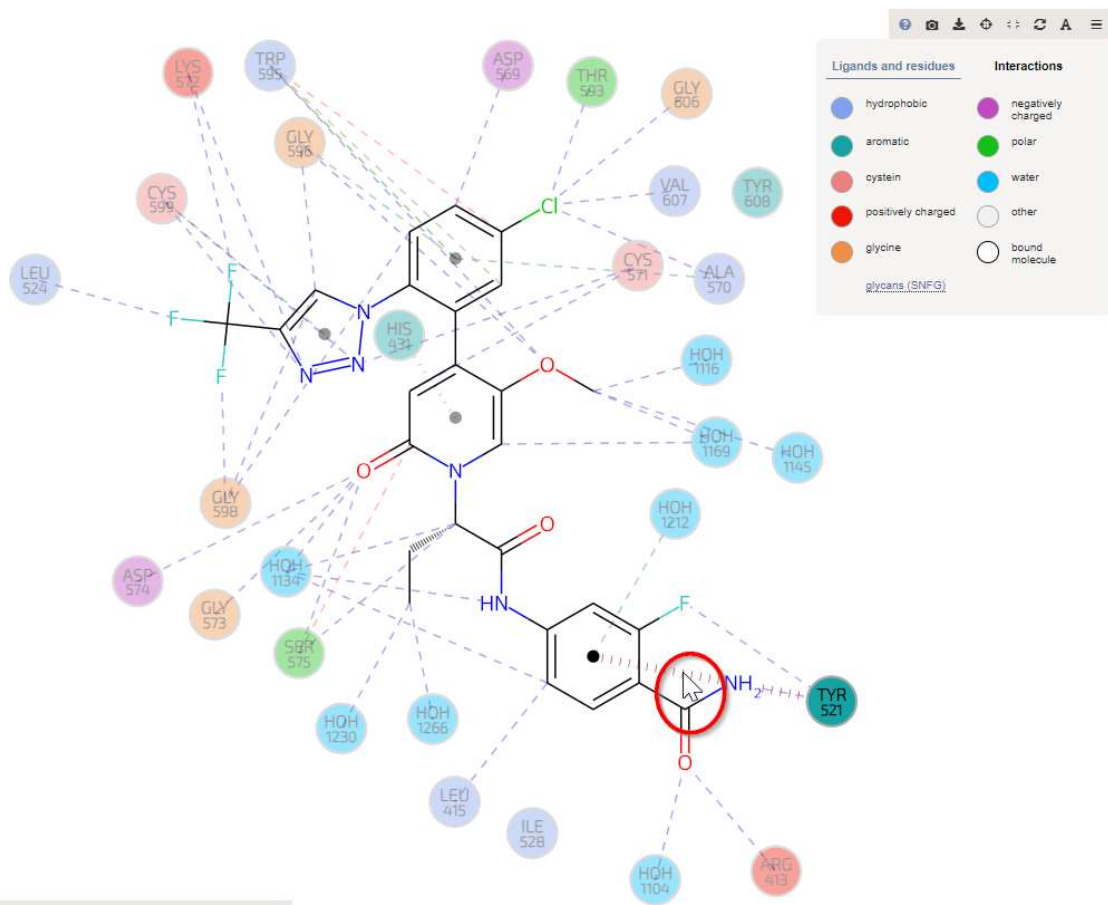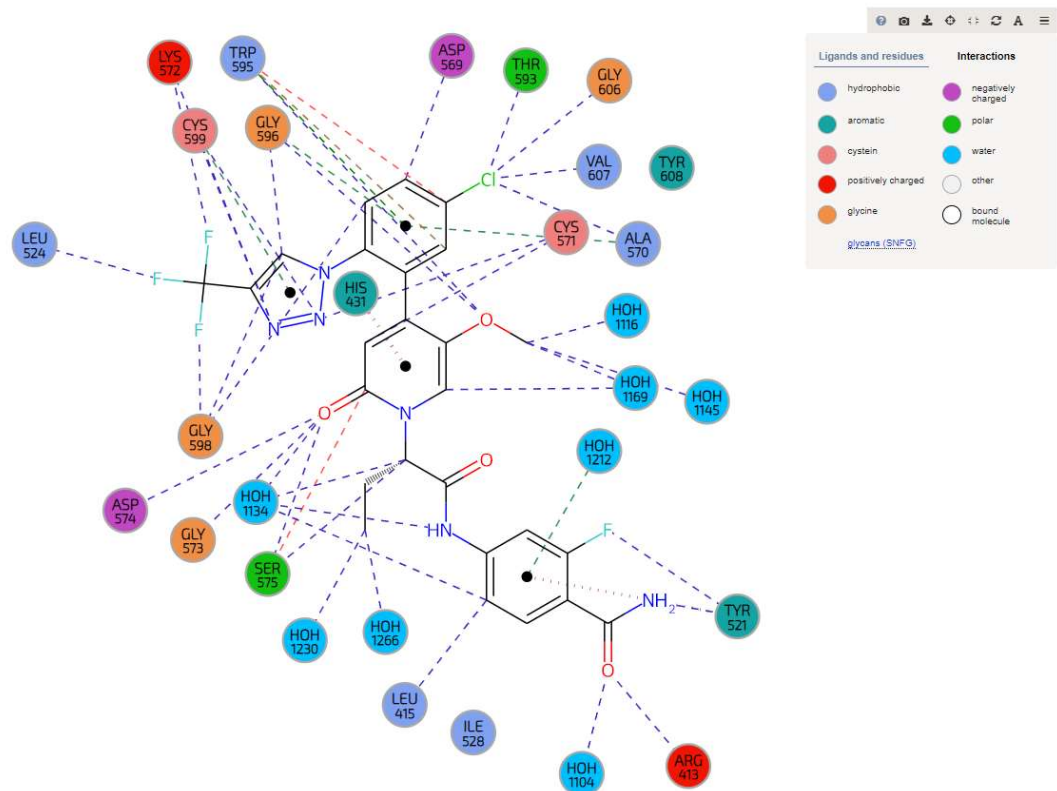
side chain interaction (CD1,CD2,CE1,CE2,CG,CZ | OE): 5.89Å

Question 5:
Can you list the total number of (a) hydrophobic, (b) positively charged, (c) negatively charged, (d) aromatic and (e) polar amino acids that are interacting directly with **Asundexian**?

- Notice how mousing over amino acids in the left panel results in the amino acid being highlighted in the right panel.
- Try interacting with the Mol* viewer on the right panel. The right panel shows the electron density that the molecule was fitted into. Notice how mousing over the amino acid in the Mol* viewer results in the highlighting in the 2D depiction.
  How well do the atoms fit into the mesh?
  Do you think molecular modelling is reliable?

- Notice how mousing over amino acids in the left panel results in the amino acid being highlighted in the right panel.
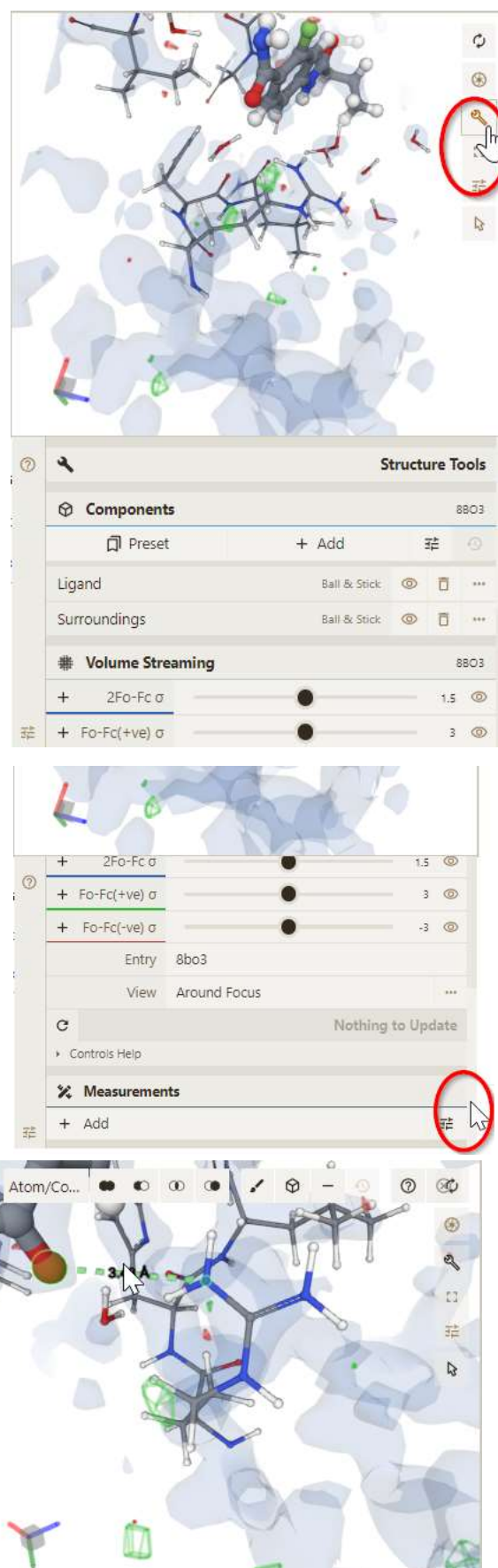
OPTIONAL:

- The right-hand side of the viewer (again) has multiple options for viewing things in different ways. Have a go playing with these options and try to do the following tasks.

  - *Move view of binding site around.* Use left and right mouse click to move round so the arginine in the binding site is the focus of the view.

  - *Add a measurement between the ligand and the binding site.* Click on the spanner. Scroll down to reveal '**Measurement**'. Click on the arrow (below spanned) and in popped-up **change from 'Residue'** to '**Atom/Coarse element**'. Select the atoms that make the hydrogen bond between the arginine and the ligand and options should appear under measurement. Select 'Distance'.

    Bonus Question 6: What is the distance between the atoms?

Ligand binding sites – part II

Viewing small molecules bound to proteins

Go to entry 8bo3 by typing "PDBe.org/8bo3" into your browser.
Click on the link under 'Primary publication'.

Take note of the 4 additional PDB ids.

Go to entry 8bo3 by typing "PDBe.org/8bo3" into your browser.
Click on the link in 'Structural analysis' block with UniProt and the special PDBe-KB icon:

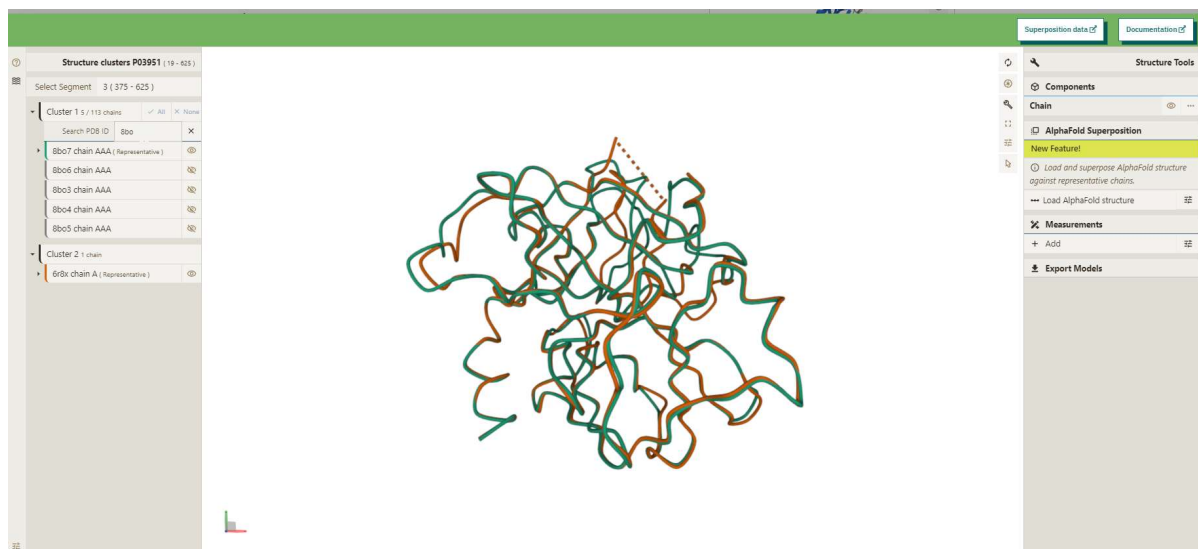https://www.ebi.ac.uk/pdbe/pdbe-kb/proteins/P03951

Select the '**3D view of superposed structure**'.

→ Go to segment 3 (adjacent '**Select Segment**').

Use the '**Seach PDB ID**' to select all ids with 8bo…

Use the eye icon so all 5 structures are visible and hide the other cluster.

Select '**Export Models**' and download a zipped file of all structures visible superposed.

Visit:
https://molstar.org/viewer/

Drop the zipped file onto viewer.

Bonus Question 7:
Can you work out which of the compounds have R-groups that occupy the same position as is occupied by glycerol in one of the structures?

Bonus Question 8:
Can you work out which of the compounds results in the arginine side chain adopting a different conformation?