

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](https://www.ebi.ac.uk/pdbe/)

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

- You should see the following header bar:

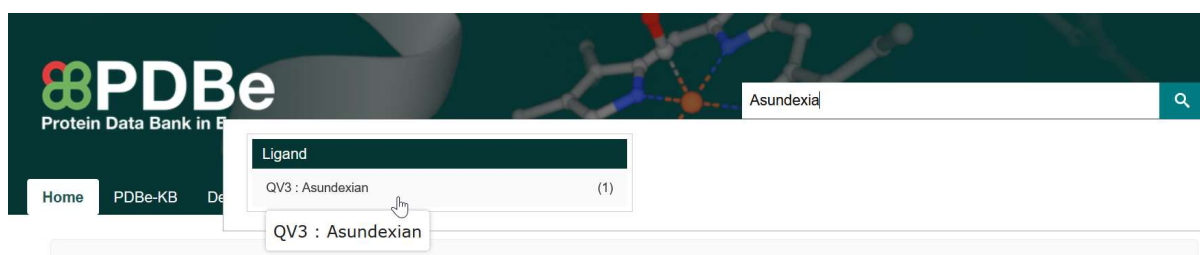


- Your search begins with the search bar at the top right.

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.

8bo3 1 COAGULATION FACTOR XI PROTEASE DOMAIN IN COMPLEX WITH ACTIVE SITE INHIBITOR Asundexian

Schaefer M, Roehrig S, Ackerstaff J, Nunez EJ, Gericke KM, Meier K, Tersteegen A, Stampfuss J, Ellerbrock P, Meibom D, Lang D, Heitmeier S, Hillisch A

J Med Chem (2023) [PMID: 37669040]

Source organism: [Homo sapiens](#)

Assembly composition: protein only structure

Bound ligands: QV3 CIT

Assembly name: Coagulation factor XIa light chain (Preferred) [search this complex](#)

PDBE complex ID: PDB-CPX-137278 (Preferred) [search this ID](#)

PDBE-KB: P03951

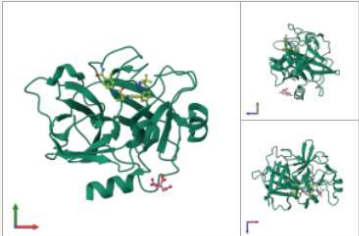
3D Visualisation 2

Download files 3

X-ray diffraction
1.841Å resolution
Released: 13 Sep 2023
DOI: [10.2210/pdb8bo3](#)

Model geometry

Fit model/data



- 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:

PDBE > 8bo3

COAGULATION FACTOR XI PROTEASE DOMAIN IN COMPLEX WITH ACTIVE SITE INHIBITOR Asundexian

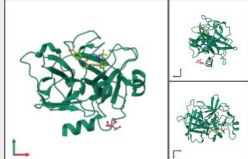
Source organism: [Homo sapiens](#)

Primary publication:
 Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders.
 Roehrig S, Ackerstaff J, Jiménez Núñez E, Teller H, Ellerbrock P, Meier K, Heitmeier S, Tersteegen A, Stampfuss J, Lang D, Schlemmer KH, Schaefer M, Gericke KM, Kinzel T, Meibom D, Schmidt M, Gerdes C, Follmann M, Hillisch A
J Med Chem (2023)
 PMID: 37669040

X-ray diffraction
1.84Å resolution
Released: 13 Sep 2023
DOI: [10.2210/pdb8bo3/pdb](#)

Model geometry

Fit model/data



Function and Biology

Reaction catalysed:
 Selective cleavage of Arg-|-Ala and Arg-|-Val bonds in factor IX to form factor IXa.

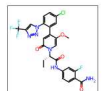
Biochemical function: not assigned


Biological process: not assigned

Cellular component: not assigned

Ligands and Environments

2 bound ligands:


 1 x QV3


 1 x CIT

No modified residues

Quick links

8bo3 overview

- Citations
- Structure analysis
- Function and Biology
- Ligands and Environments
- Experiments and Validation

View

Downloads

3D Visualisation

Citations

1 mention without citation

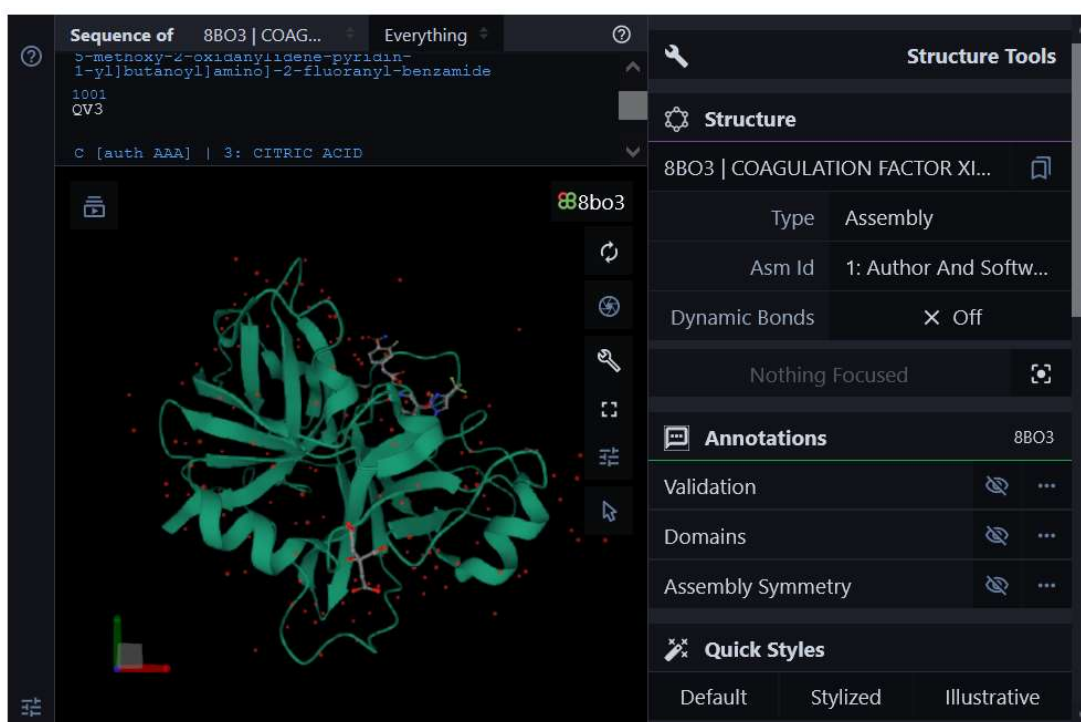
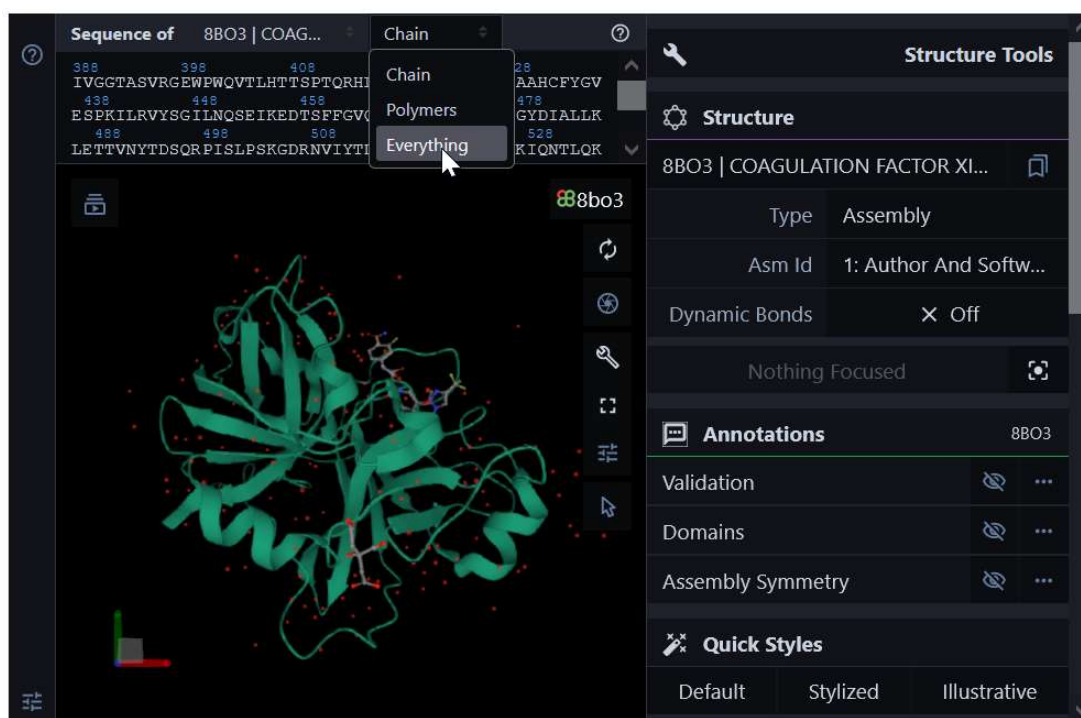
Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders.
 Roehrig et al. (2023)

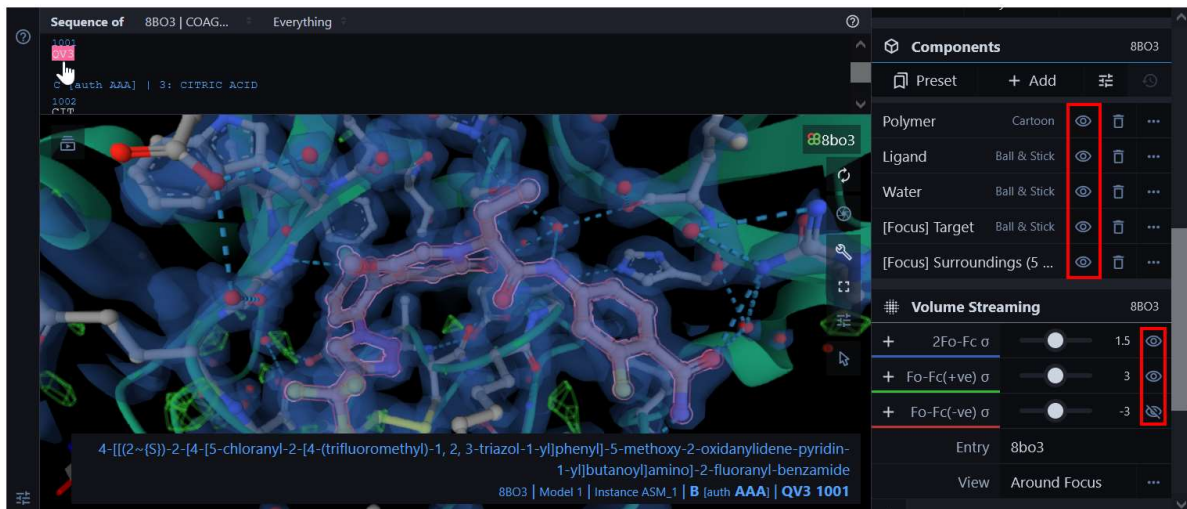
- The first thing we will do is view the structure in 3D using Mol*
 - click ‘3D Visualisation’ highlighted in the red box above

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:
 - Polymer** – 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?

*ANSWER: cartoon representation
– highlights secondary structure*

- Ligand** – 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:

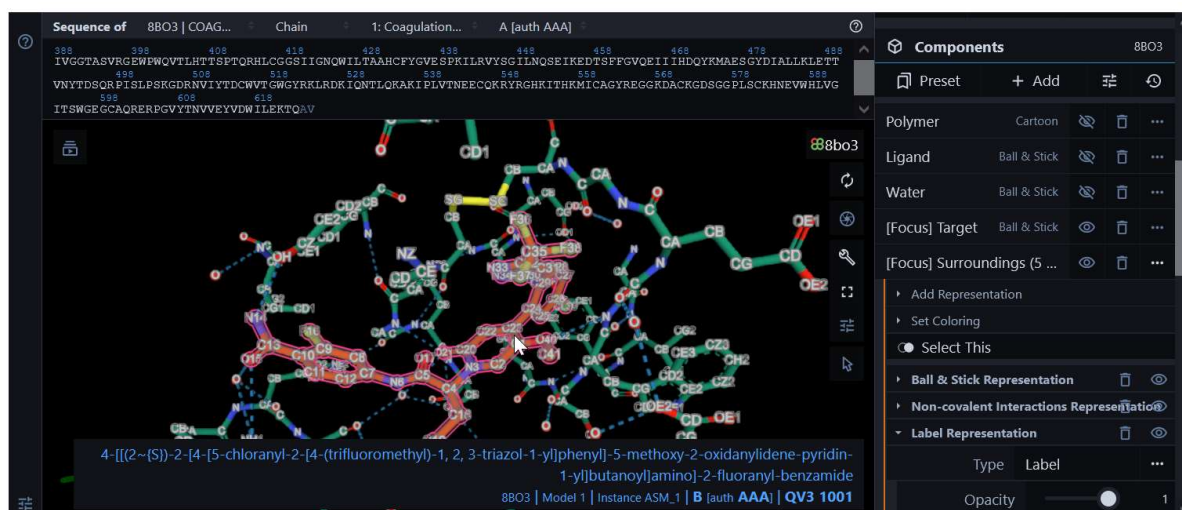
Can you work out which elements are involved in the inhibitor in this structure?
(Hint: we usually don’t show hydrogens on the molecule)

ANSWER: Carbon (grey), Nitrogen (blue), Oxygen (red), Fluorine (green), Chloride (green), Hydrogen (not shown)

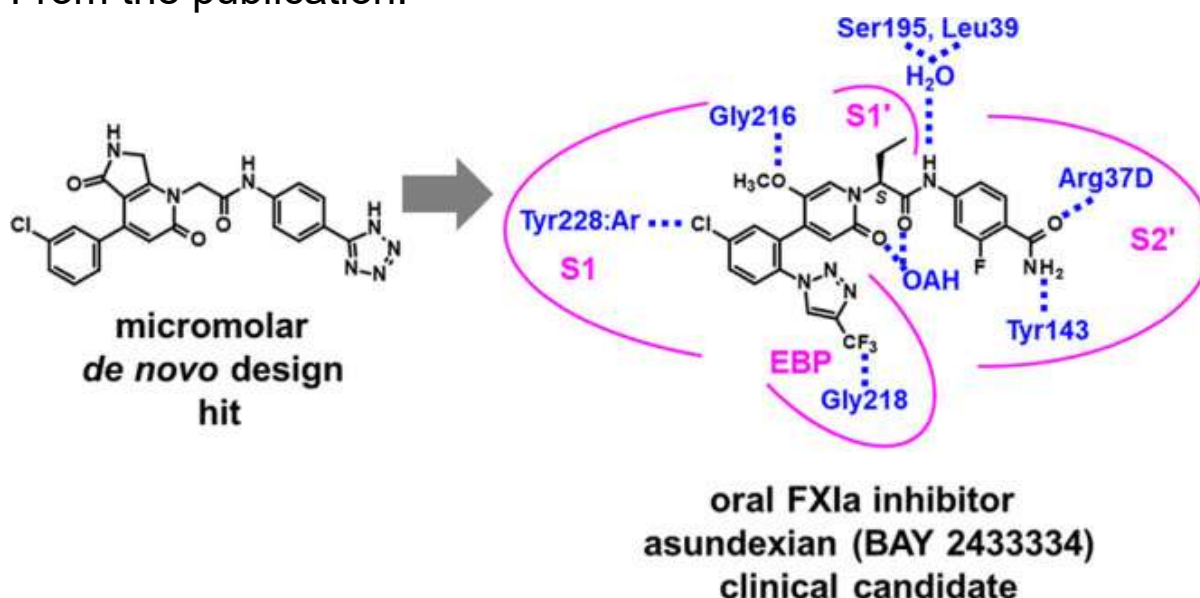
- **Water** – Most PDB structures contain water molecules as proteins prefer to be hydrated. Therefore, individual water molecules are also found in the structure.
- **[Focus] Target** and **[Focus] surrounding (5A)** – this only appears upon clicking on a ligand.

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 it to '**Element**'.



From the publication:



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

Journal of Medicinal Chemistry **2023** 66 (17), 12203-12224

DOI: 10.1021/acs.jmedchem.3c00795

<https://europepmc.org/article/MED/37669040>

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?

ANSWER:

Atom N6 from Asundexian forms a water mediated hydrogen bond with two amino acids serine and leucine.

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 PDB.org/8bo3
- The small molecules in the PDB entry are displayed in the 'Ligands and Environments' section (in the red box below).

The screenshot shows the PDB entry page for 8bo3. The main title is 'COAGULATION FACTOR XI PROTEASE DOMAIN IN COMPLEX WITH ACTIVE SITE INHIBITOR Asundexian'. The source organism is *Homo sapiens*. The primary publication is 'Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders' by Roehrig S, Akerstaff J, Jiménez Núñez E, Teller H, Ellerbrock P, Meier K, Heitmeier S, Tersteegen A, Stampfuss J, Lang D, Schlemmer KH, Schaefer M, Gericke KM, Kinzel T, Meiborn D, Schmidt M, Gerdes C, Follmann M, Hillisch A, published in *J Med Chem* (2023) with PMID: 37669040.

The 'Ligands and Environments' section, highlighted with a red box, shows two bound ligands: 1 x QV3 and 1 x CIT. Below this, it states 'No modified residues'.

The 'Function and Biology' section indicates that the protein catalyzes the 'Selective cleavage of Arg-[-Ala and Arg-[-Val bonds in factor IX to form factor IXa'.

The 'Quick links' sidebar includes links for Citations, Structure analysis, Function and Biology, Ligands and Environments, Experiments and Validation, View, Downloads, and 3D Visualisation.

The 'Citations' section shows one mention without citation: 'Design and Preclinical Characterization Program toward Asundexian (BAY 2433334), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders, Roehrig et al. (2023)'.

- 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.

EMBL-EBI Protein Data Bank in Europe
Bringing Structure to Biology

8bo3 > QV3

4-[[[(2-{S})-2-[4-[5-chloranyl-2-[4-(trifluoromethyl)-1,2,3-triazol-1-yl]phenyl]-5-methoxy-2-oxidanylidene-pyridin-1-yl]butanoyl]amino]-2-fluoranyl-benzamide

Formula: C₂₆ H₂₁ Cl F₄ N₅ O₄

Molecular weight: 593 Da

Environment details **NEW**

QV3 1001 bound to chain AAA

Quick links

- 8bo3 overview
- Citations
- Structure analysis
- Function and Biology
- Ligands and Environments**
- Experiments and Validation

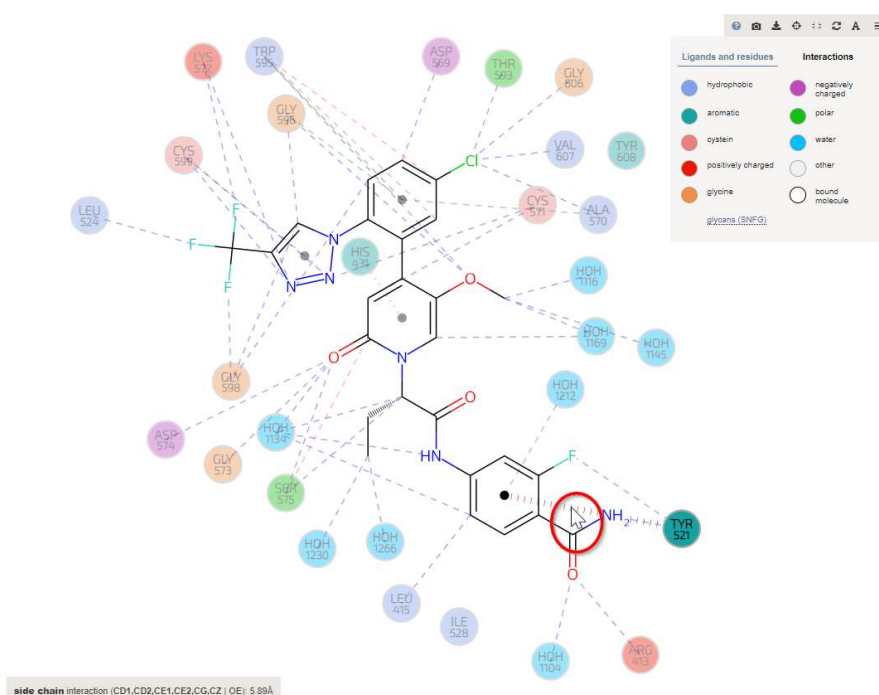
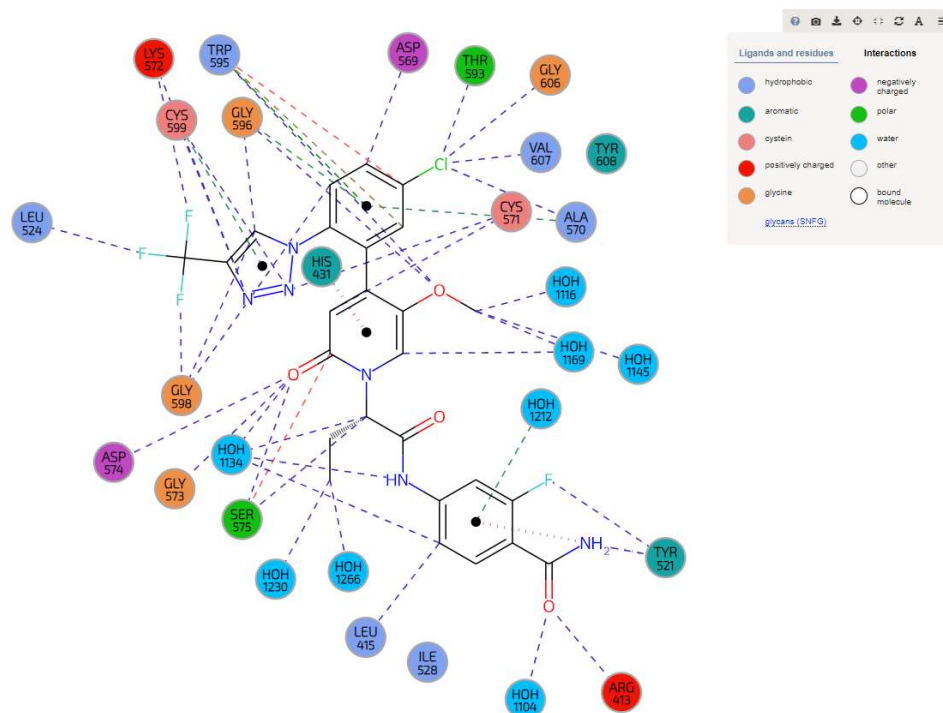
Downloads

EBI resources (QV3)

- QV3 in PDBeChem

- 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'?)



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**?

ANSWER: (a) 6 hydrophobic, (b) 2 positively-charged, (c) 2-negatively-charged, (d) 2 aromatic, (e) 2 polar amino acids

- 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?

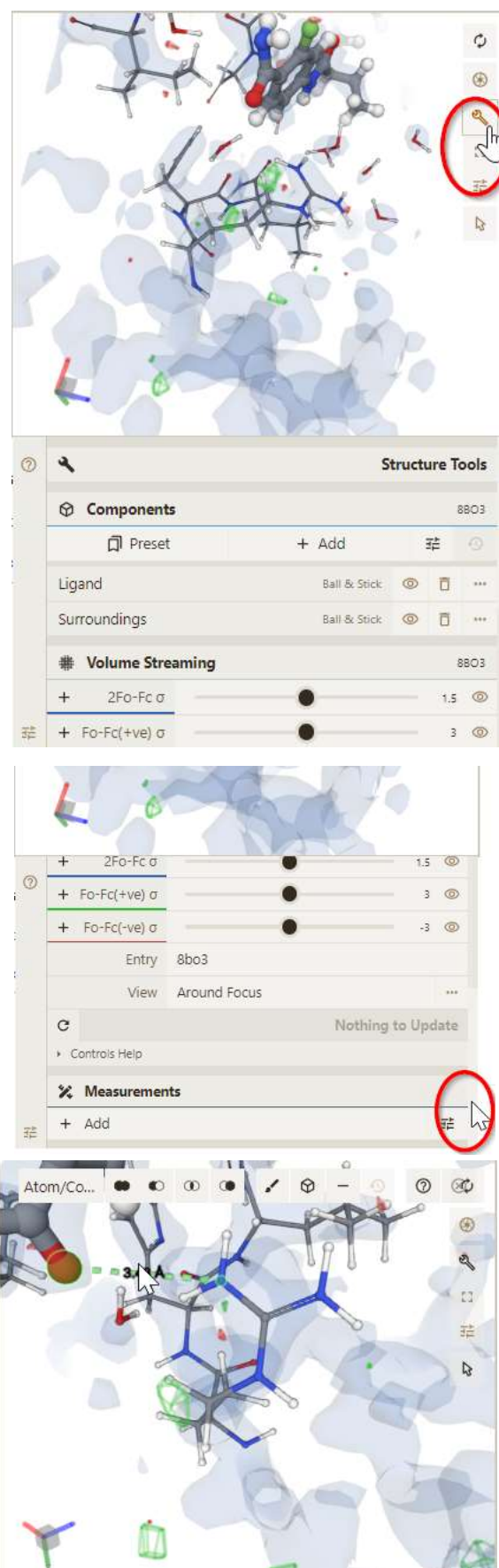
- Notice how mousing over amino acids in the left panel results in the amino acid being highlighted in the right panel.

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?
ANSWER:
3.48 Angstrom



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.

PDBe > 8bo3

COAGULATION FACTOR XI PROTEASE DOMAIN IN COMPLEX WITH ACTIVE SITE INHIBITOR Asundexian

Source organism: *Homo sapiens*



Primary publication:
[Design and Preclinical Characterization Program toward Asundexian \(BAY 2433334\), an Oral Factor XIa Inhibitor for the Prevention and Treatment of Thromboembolic Disorders.](#)

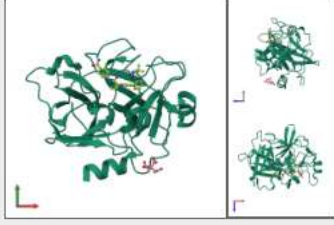
Roehrig S, Ackerstaff J, Jiménez Núñez E, Teller H, Ellerbrock P, Meier Heitmeier S, Tersteegen A, Stampfuss J, Lang D, Schlemmer KH, Schaefer M, Gericke KM, Kinzel T, Meibom D, Schmidt M, Gerdes C, Follmann M, Hillisch A


J Med Chem (2023)
PMID: 37669040

X-ray diffraction
1.84Å resolution

Released: 13 Sep 2023
DOI: [10.2210/pdb8bo3/pdb](https://doi.org/10.2210/pdb8bo3/pdb)


Model geometry 
Fit model/data 





Function and Biology  Details

Reaction catalysed:

Selective cleavage of Arg-|-Ala and Arg-|-Val bonds in factor IX to form factor IXa.

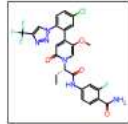
Biochemical function:  not assigned

Biological process:  not assigned

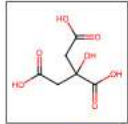
Cellular component:  not assigned

Ligands and Environments

2 bound ligands:



[1 x QV3](#)



[1 x CIT](#)

No modified residues

Go to entry 8bo3 by typing “PDBe.org/8bo3” into your browser.

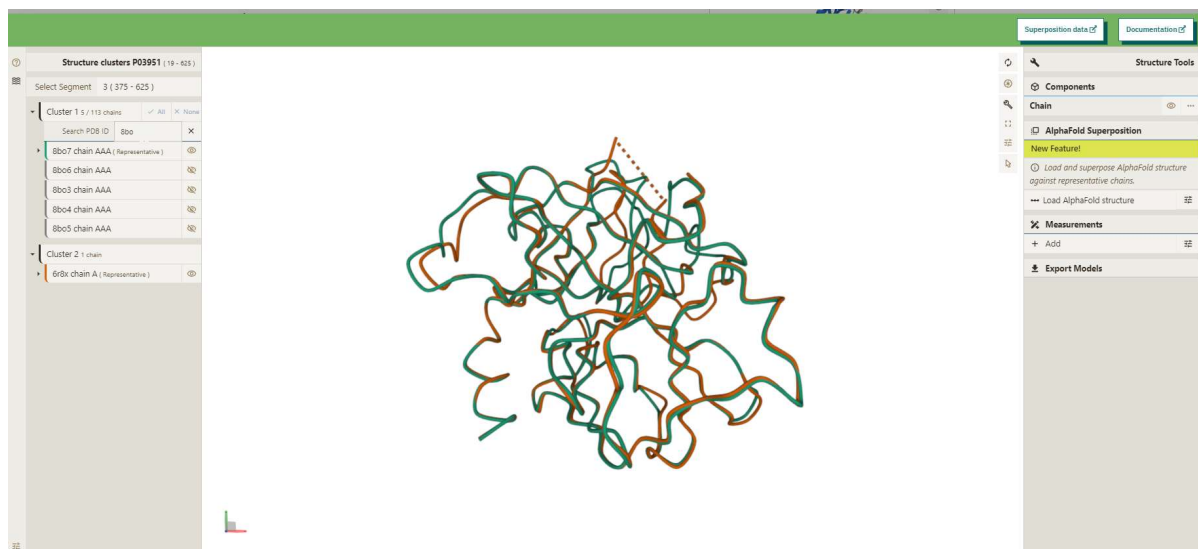
Click on the link in ‘Structural analysis’ block with UniProt and the special PDBe-KB icon:



<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 '**Search 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:

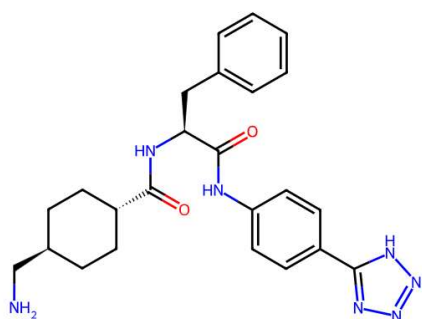
<https://molstar.org/viewer/>

Drop the zipped file onto viewer.

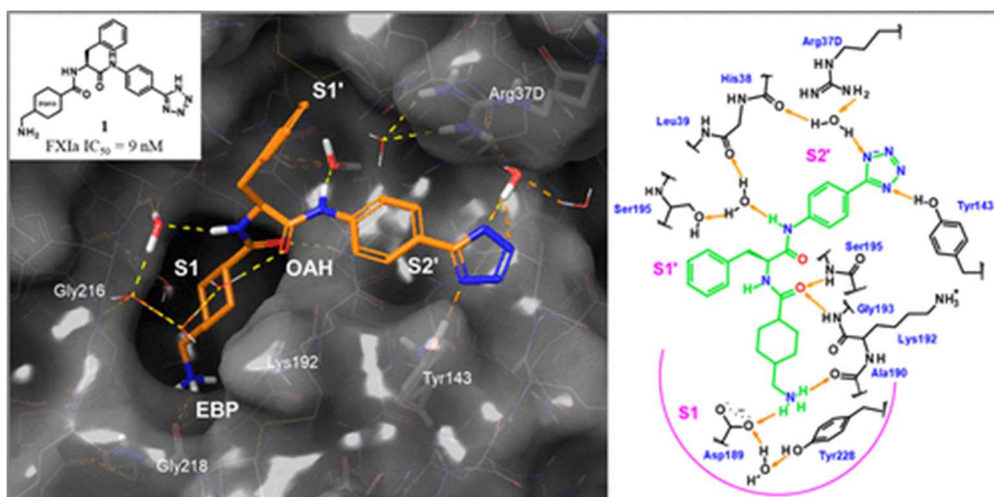
Bonus Question 7:

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?

ANSWER:



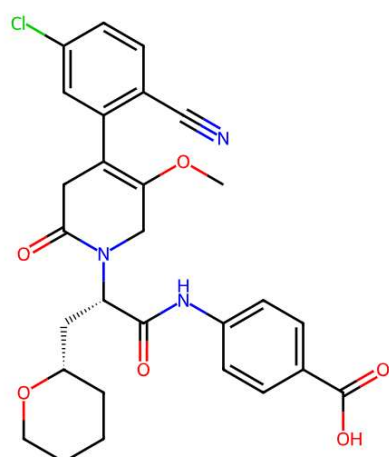
CC ID: QVI



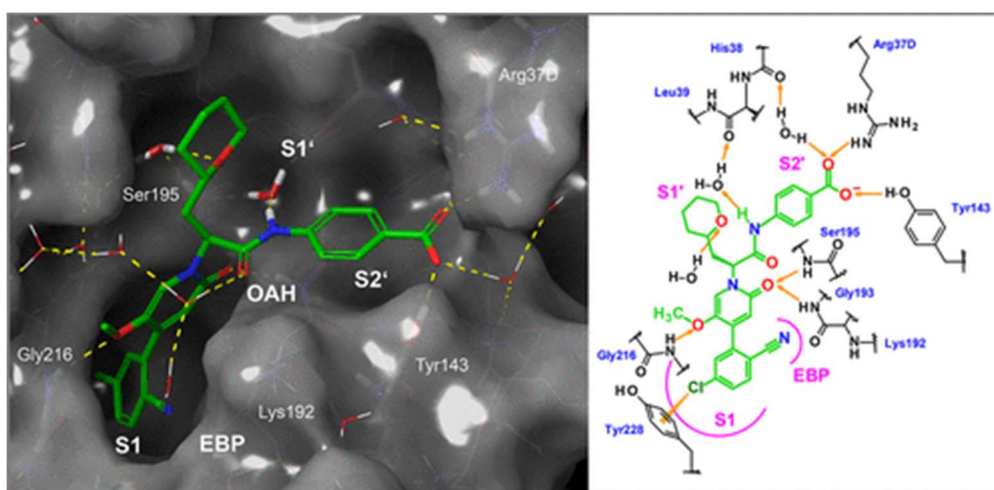
Quote from the publication:

The low membrane permeability observed made it an unfavorable starting point for our program.

And



CC ID: QW6



Quotes from the publication:

At that point in the project, carboxylic acid **34** was regarded as the best compound overall and was profiled in depth: **34** inhibited human FXIa directly, reversibly, potently, and selectively with an IC_{50} of 0.5 nM in buffer.

With additional favorable results in preclinical safety studies, **34** was chosen for evaluation in a first-in-human study. In this trial, however, **34** turned out to have lower oral exposure and a shorter dominant half-life in humans than expected.

Bonus Question 8:

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

ANSWER:

CC ID: QW6

