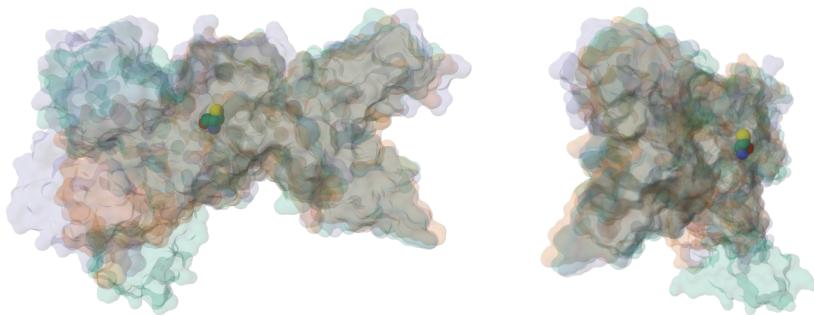




## Guide on how to determine whether a residue is ‘buried’, or at the surface of its protein chain, using PDBe-KB superposition.

The final image from following the steps is:



### Summary of variant information related to the example:

UniProt indicates ID O60260 has a variant C289G, that is associated with **Parkinson Disease 2 (PARK2) [MIM:600116]**, publications on this variant indicate it results in:

- increased aggregation
- fails to ubiquitinate SYT11
- loses ability to bind SYT11
- impaired relocalization to damaged mitochondria
- loss of function in mitophagy

**Visualising the protein’s 3D structure using [Mol\\* viewer](#) helps assess whether an amino acid is buried or at the surface of the protein.**

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### Getting PDBe-KB superposition

The below is an example superposition from the PDBe-KB ‘Structures’ page.

The example superposition in the images is from:

<https://www.ebi.ac.uk/pdbe/pdbe-kb/proteins/O60260/structures>

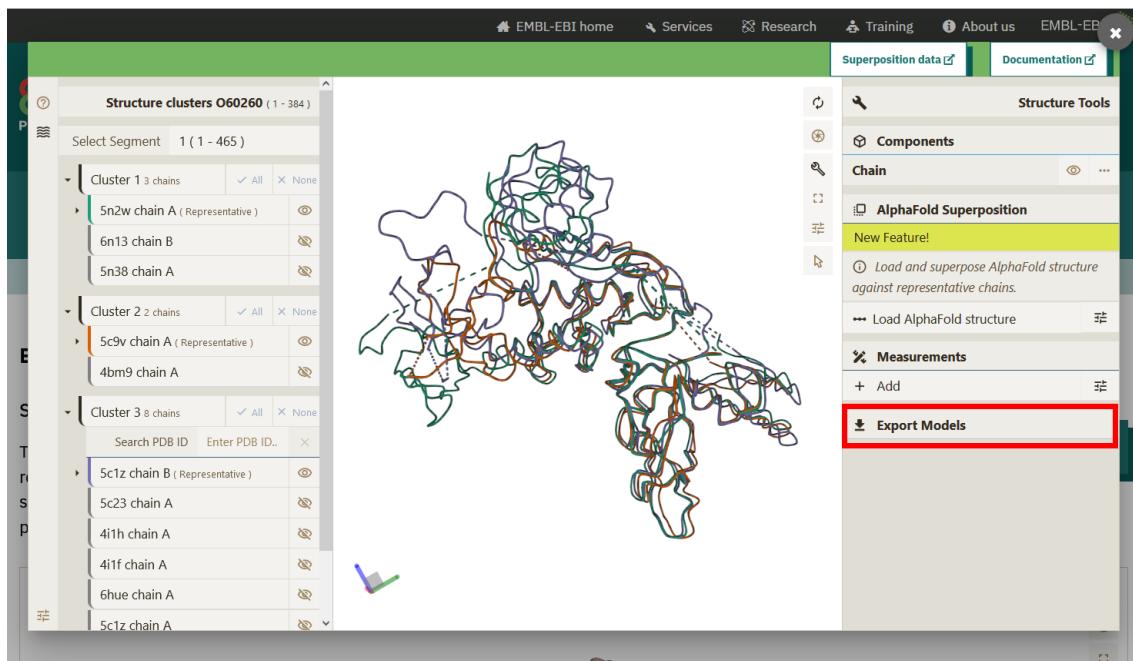


Figure 1. Superposition view from PDBe-KB ‘Structures’ webpage

Clicking on the ‘Export Models’ button (red rectangle in **Figure 1**) will download a superposition with the visible chains (i.e. chains with eye  icons not crossed-out).

These will download as zipped folder that can be dropped into [Mol\\* viewer](#).

The superposition utilises single protein chains from experimentally determined structures that belong to a specific UniProt ID (e.g. O60260), so each instance listed in the left panel are single chains, even if there might be multiple chains in the experimentally determined structures.

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### **Working in ‘selection’ mode in mol\***

#### Step 1:

‘Drop’ the PDBe-KB superposition zipped folder onto the [mol\\* viewer](#).

The load objects appear on the left (red box in **Figure 2**). Each of the ‘.cif’ objects represents a different chain that has been extracted from the experimentally determined coordinates as part of PDBe-KB superposition.

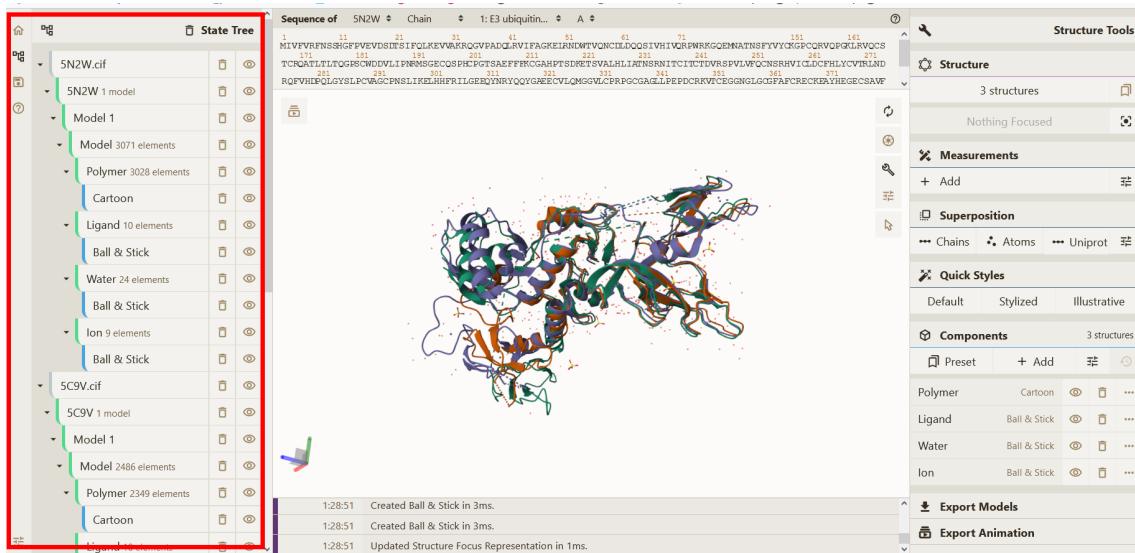


Figure 2. Mol\* viewer after the superposition has been loaded

#### Step 2:

Start **Selection mode** by clicking on the arrow icon (red circle in **Figure 3**).

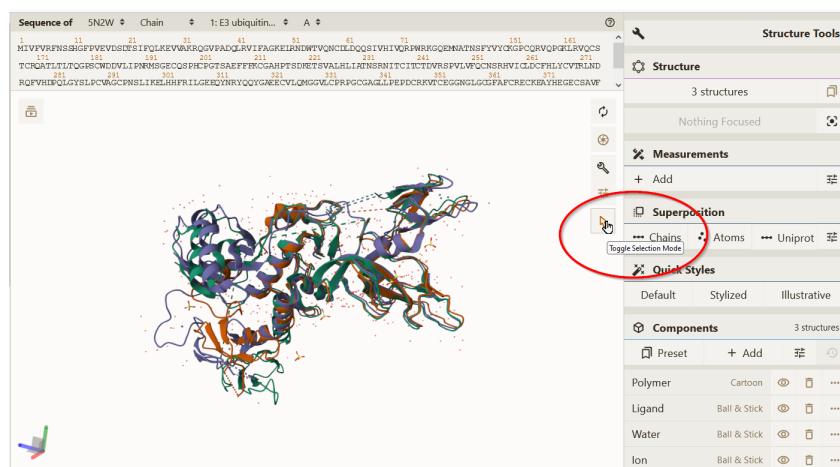


Figure 3. Mol\* viewer currently in ‘Default mode’

After clicking on a new menu will appear as in **Figure 4** (red box).

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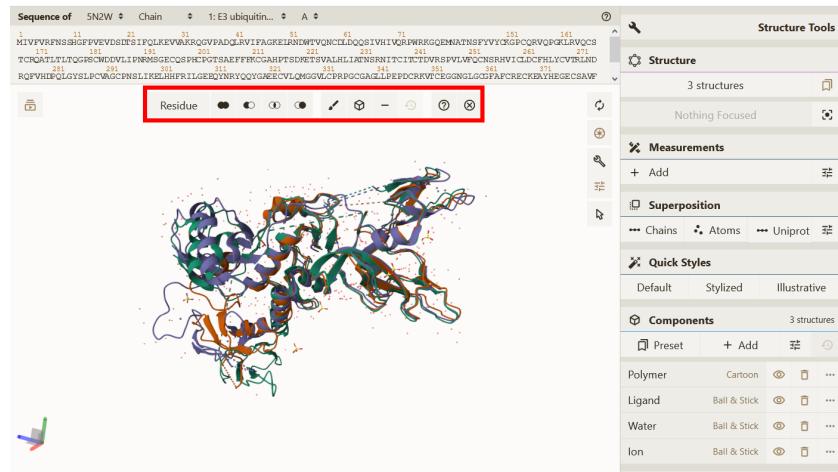


Figure 4. Mol\* viewer currently in 'Selection mode'

### Step 3:

The sequence panel can be used to find the relevant position in the protein sequence.

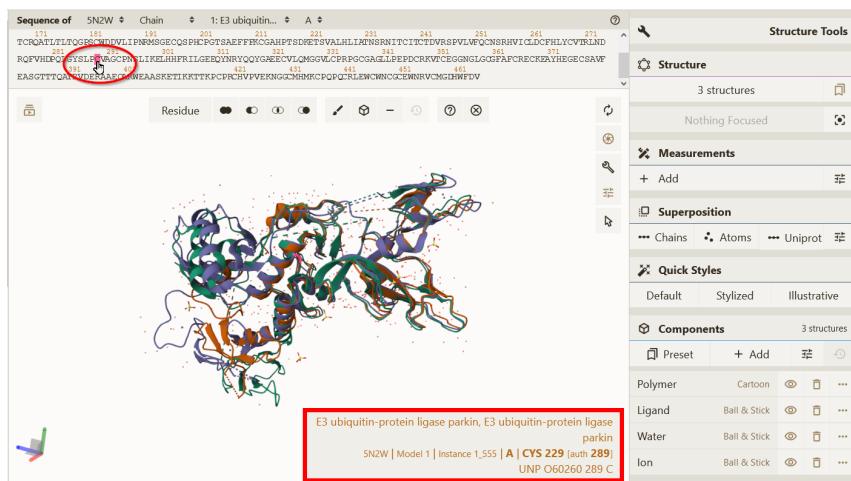


Figure 5. Mol\* viewer when interacting with top panel containing sequences etc

Mousing over residues in the sequence (red circle in **Figure 5**) causes a box to appear in the central 3D canvas (red box in **Figure 5**).

If the variant position cannot be found, interact with the components in the sequence panel. The scroll bar (red circle in **Figure 6**) will enable access to parts of sequence not displayed. To access other chains ids, proteins, or coordinates use the other interactive components (red boxes in **Figure 6**).

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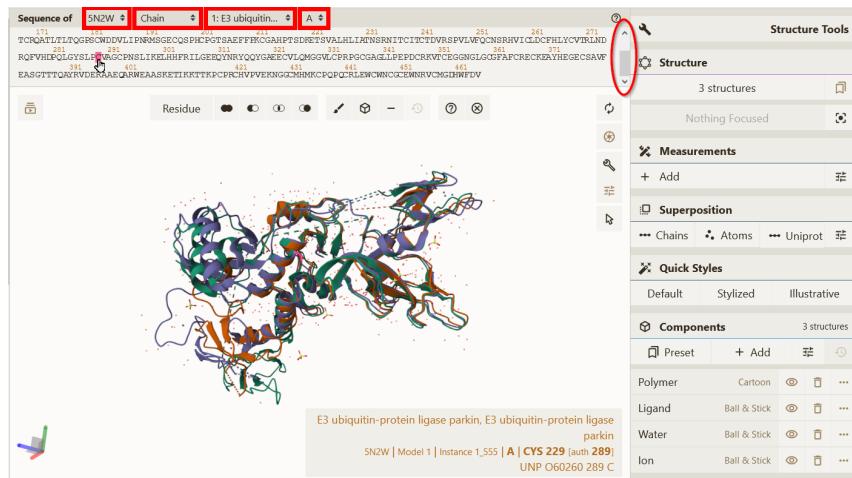


Figure 6. Interactive components in the Top (sequence) panel

### Step 4:

Click-on the sequence at relevant position (e.g. Cys289). This generates green highlighting and '1 Residue Selected' appears on Left (control) panel.

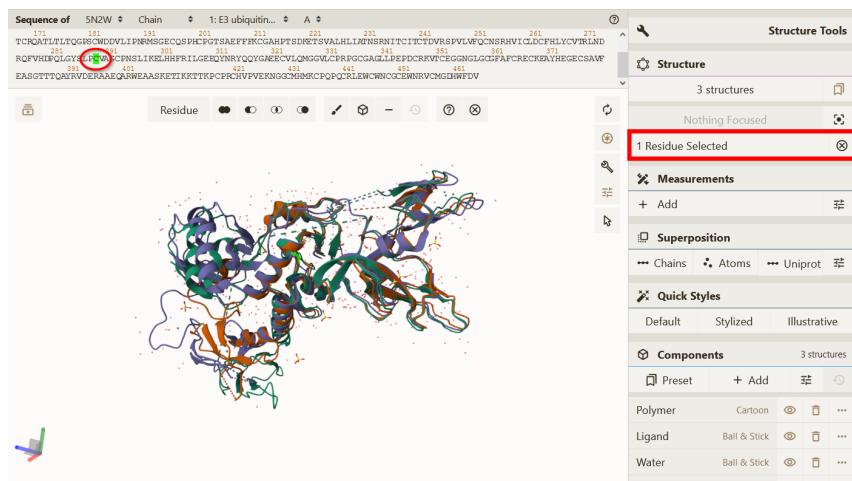


Figure 7. Mol\* viewer with a single residue selected

This selection will be converted to a component.

### Step 5:

To convert the selection to a component, click-on the cube icon

This will open a new panel with **Add Component** at the top.

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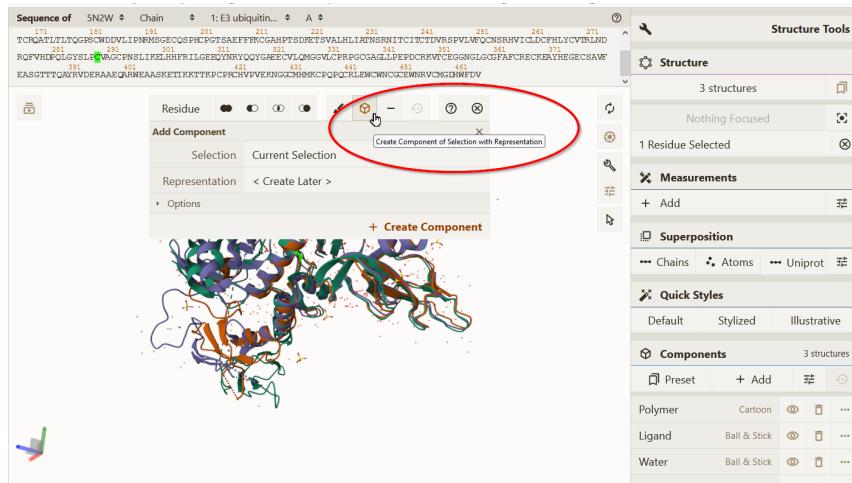


Figure 8. Add component (from selection) panel opened

To add a name to the new component, click-on **Options** (red circle in **Figure 9**) and type the name in text box adjacent to **Label** (red box in **Figure 9**).

Then click-on **Create Component**.

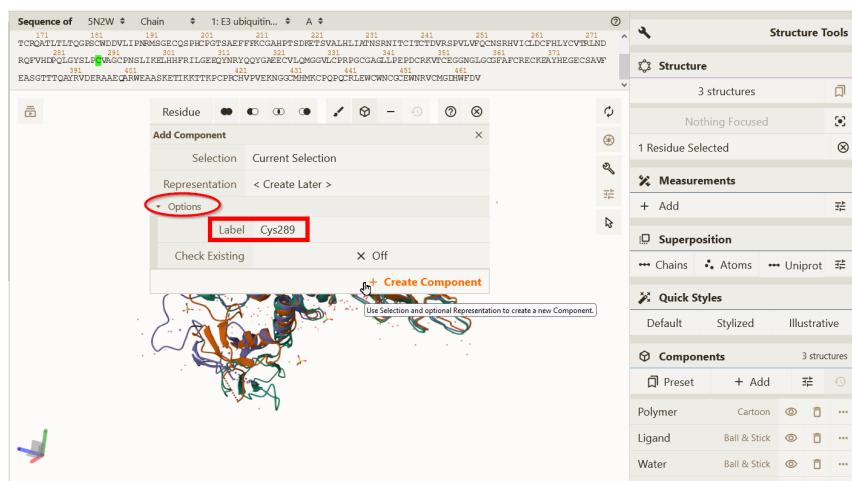


Figure 9. Interacting with the 'Create component' (from selection) panel

### Step 6:

After the component has been created, the 'Selection mode' can be turned off (red circle in **Figure 10**). To see the new component, it may be necessary to use the outermost scroll bar (red arrow in **Figure 10**).

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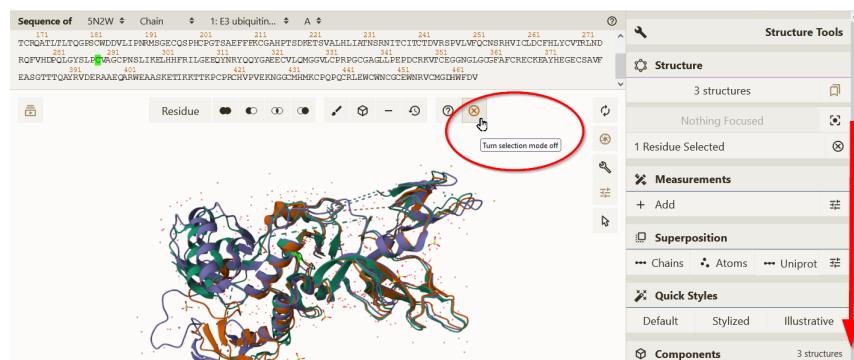


Figure 10. Turn off the 'Selection mode' and use outer scroll bar to see new component

## Working with components & representations in mol\*

### Step 7:

The new component when moused over (red box in **Figure 11**) will result in pink highlighting on the relevant residue (red circle in **Figure 11**).

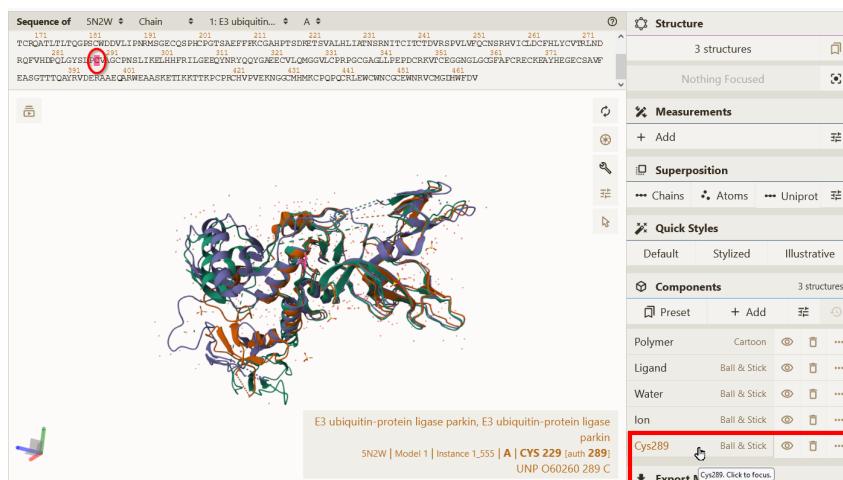


Figure 11. Mol\* viewer with a new component

Click-on the 3 dots (...) adjacent (red circle in **Figure 12**) to the new component. After this click-on **Add Representation**, and then click-on **Ball & Stick Representation**. This will result in a new Representation for the residue of interest (red box in **Figure 12**).

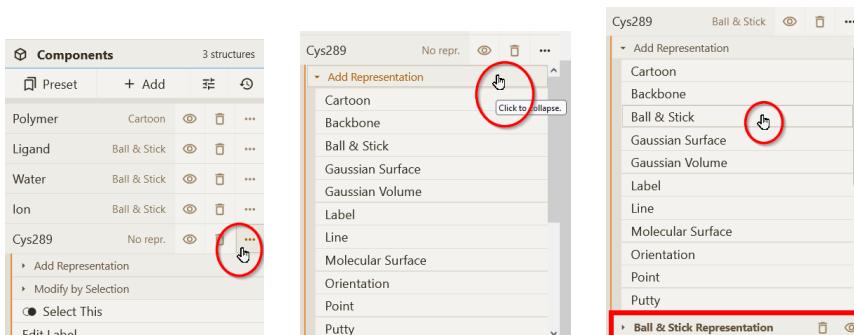


Figure 12. Navigating the component 'Add Representation' options.

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### Step 8:

There is now a Representation that can act as a reference while considering if the amino acid is buried or on the surface of the protein.

The three dots next to the new component can be used to hide the many options for Representation etc.

Clicking-on the new component will zoom-in on the residue (red circle in **Figure 13**). Alternatively, clicking-on the residue in the Top (sequence) panel will zoom-in on the residue (red box in **Figure 13**).

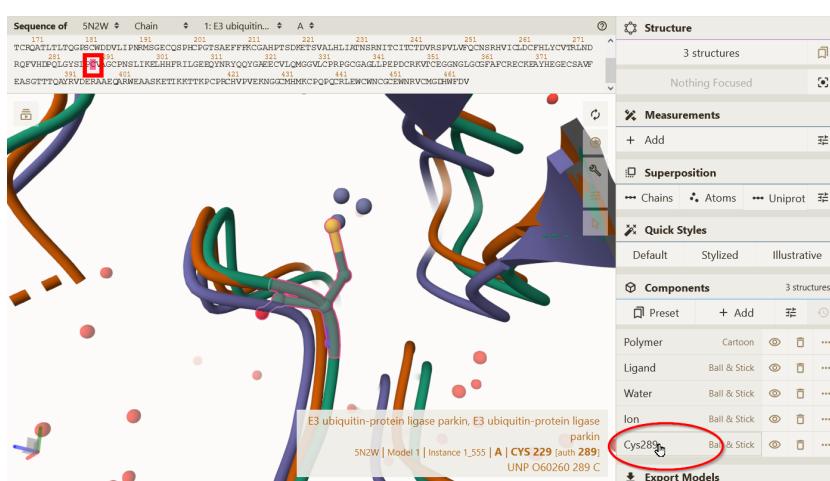


Figure 13. Zooming-in on residue of interest

Clicking on the ‘Reset zoom’ button (red circle in **Figure 14**), will return zoomed-out view.

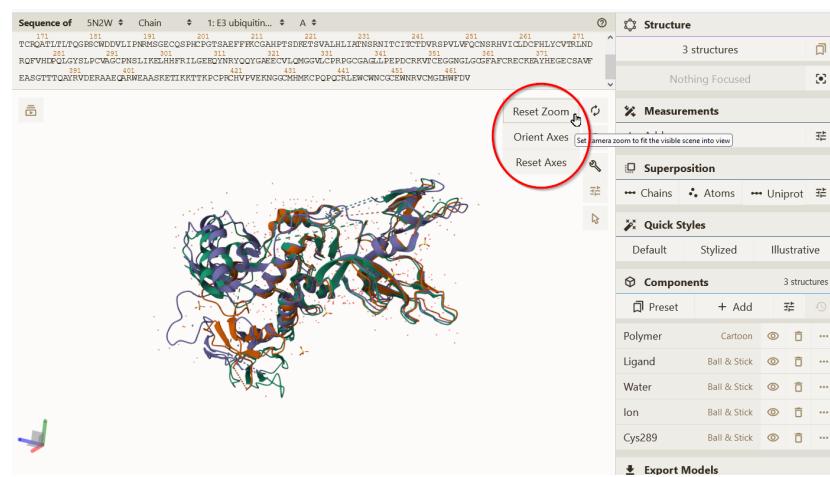


Figure 14. Zoomed-out view

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## Step 9:

Click-on the 3 dots (...) adjacent (red circle in **Figure 15**) to the ‘*Polymer*’ component. The red arrows in **Figure 15** indicate that this is the ‘*Components*’ section and that ‘*Polymer*’ is the first in the list of components.

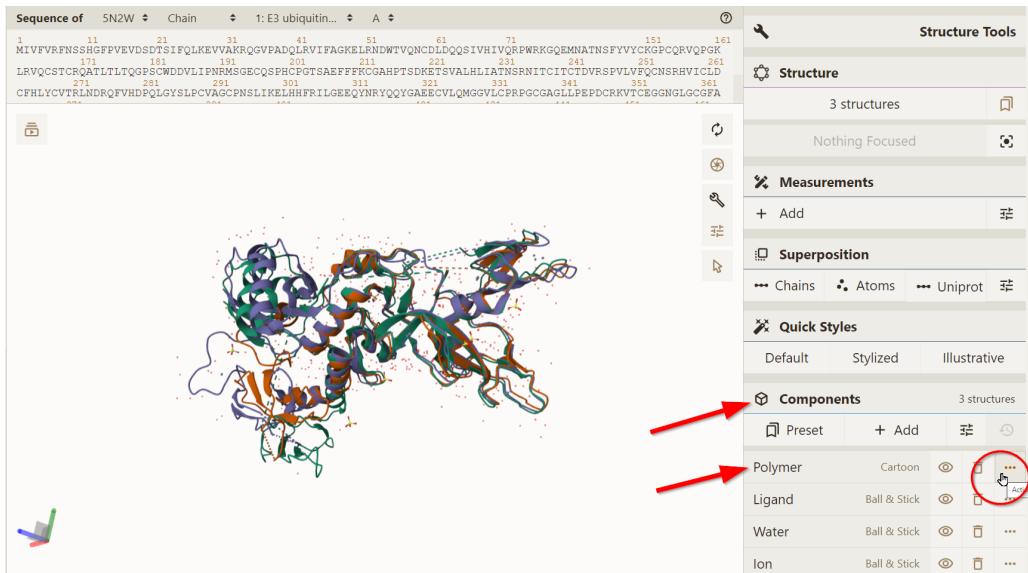


Figure 15. Mol\* viewer focusing on the ‘components’ section

After the 3 dot (...) a new menu appears, with the option **Add Representation**. Click-on **Add Representation** and scroll through the options (red arrows in **Figure 16**) and then click-on one or more of the following:

- **Gaussian Surface**
- **Gaussian Volume**
- **Molecular Surface**
- **Spacefill**

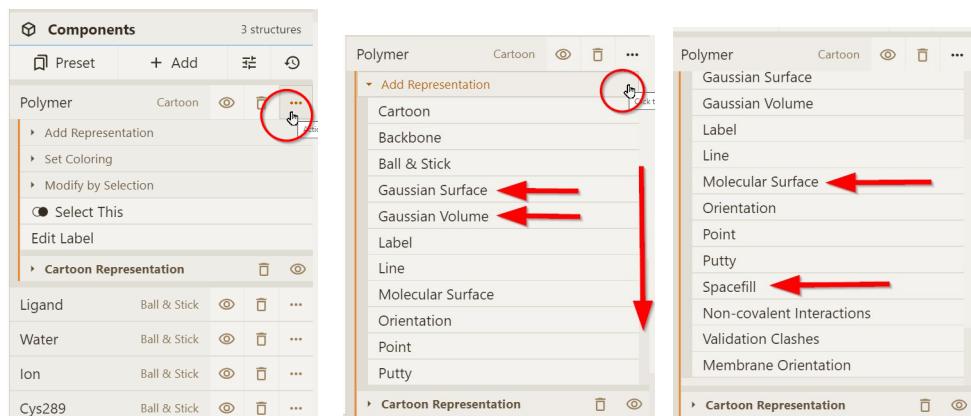


Figure 16. Navigating to make more Polymer Representations

Each of these Representations of the 3D macromolecular structure can aid in visualisation of surface vs. buried amino acids.

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## Interpreting the effects of genetic variants on protein structure and function

### Step 9:

Once the Representations are added, they can be toggled off & on by clicking on the eye  icons (red circle in **Figure 17**).

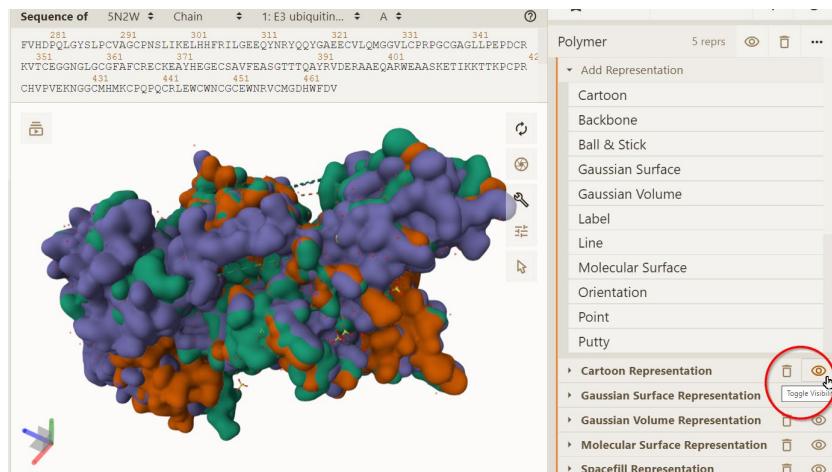


Figure 17. Turning-off a Representation

To let's try turning all the Representations off for the '**Polymer**' except the **Molecule Surface Representation**.

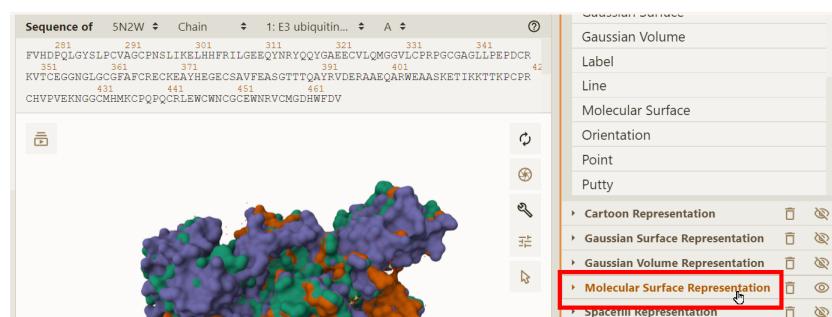


Figure 18. Opening the options for a Representation

Click-on Representation name (red box in **Figure 18**) to access the options. After this click-on the 3 dots (...) adjacent to 'Type.' Representation name and this opens even more options, including transparency (called 'Opacity') (red boxes in **Figure 19**). Default is 1, which means 100%. To decrease to 20%, 0.20 can be typed followed by pressing enter. Clicking on the 3 dots (...) adjacent to 'Polymer' to close all the options (red box in **Figure 20**).

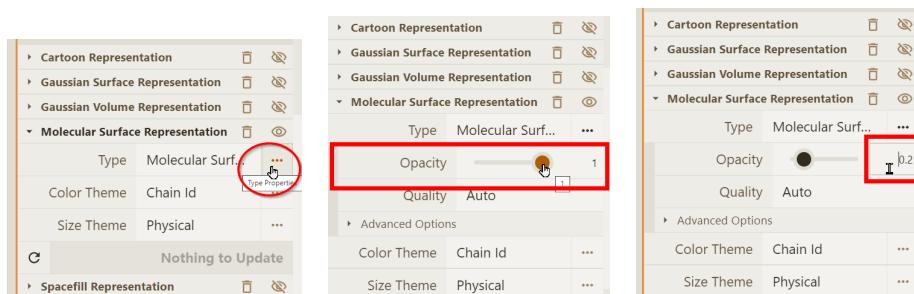


Figure 19. Making a Representation semi-transparent

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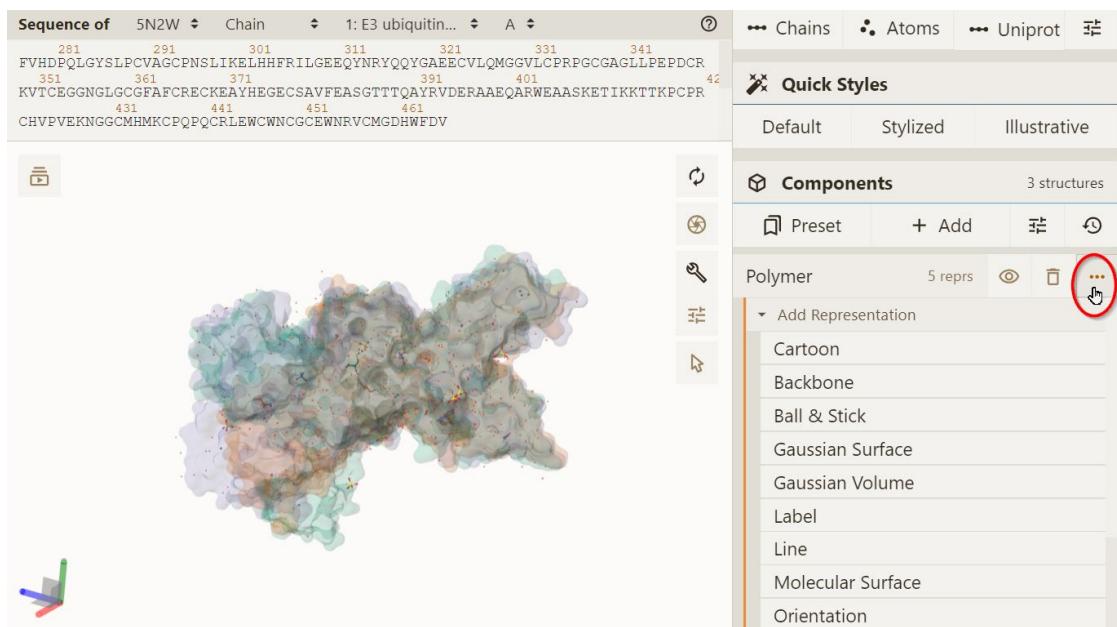


Figure 20. Closes the Component options

### Step 10:

#### Final steps:

Click on the eye icons for: ‘**Ligands**’, ‘**Water**’, and ‘**Ion**’ to hide these components. Also, lets added a **Spacefill Representation** for the ‘new’ component that contains the variant residue – in the example named ‘Cys289’.

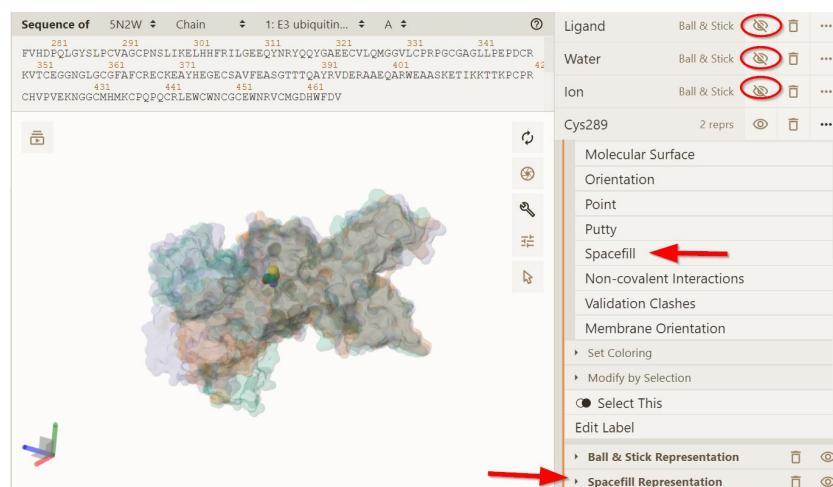


Figure 21. Final steps: hide components and add Spacefill Representation

Explore hiding and showing the other Representation and/or components.

It is possible another Representation will give different insight or a better view.