Visualizing 3D Protein Structures

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In this tutorial, you will work with a 3D structure and use the various controls in Geneious to view the features of the molecule.

Protein structure

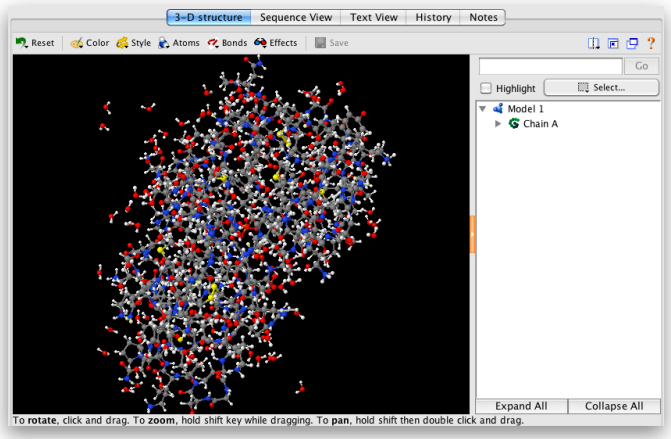
Proteins seen in nature have evolved to perform specific functions. Functional properties are dependent on their three-dimensional structures which arise because particular sequences of amino acids in linear polypeptide chains (primary structure) fold to generate small elements of secondary structure (β-sheets, α-helices and loops). These combine to form compact domains with specific three-dimensional structure (tertiary structure) which can in turn join with other polypeptide chains to create large multimers (quaternary structure). The three-dimensional structure brings together the various amino acids that form the functional region or active site.

You can get more detail on protein structure at:
http://en.wikipedia.org/wiki/Protein_structure

In this tutorial, you will work with Bovine Ribonuclease A.

Viewing the 3D structure

The PDB data for Bovine Ribonuclease A (**5RSA**) is provided. Open the document and you should be presented with a view like this:



This isn't a static image and the molecule can be moved around by clicking in the window and dragging the mouse while holding the left mouse button down.

You can also zoom the structure in and out by clicking in the window and holding both the left mouse button and ⇧**Shift** key on your keyboard down. Moving the mouse up will make the structure appear smaller as your point of view moves away. Moving the mouse down will make the structure grow as your point of view moves towards it. Moving the mouse left or right will rotate the structure in the appropriate direction.

Finally, you can pan the structure by holding the ⇧**Shift** key down and double clicking in the window and holding the mouse button down. Now as you move the mouse around the structure will pan in the window.

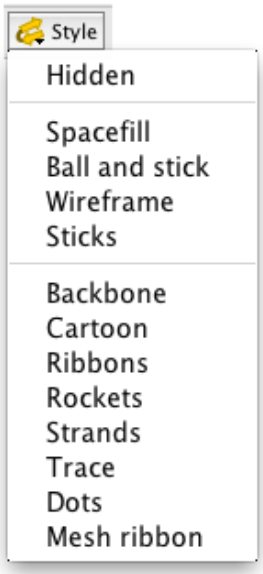
Try all of these and get used to moving the structure around. One thing you might notice is that when you are rotating the structure, it looks more three-dimensional than when it is static. This is a well known visual effect and is commonly used to help show depth on a 2D display. Gently rocking the structure back and forth will allow you to more easily see which parts are at the back and which at the front.

Simplifying the view

As you have probably realised, it is difficult to see very much with this default (ball and stick) view of the structure. For this reason, Geneious provides a number of tools that manipulate the display.

Styles

Click on the **Style** button and you will be offered a number of alternatives. Each applies to the selected atoms only but for the moment all atoms are selected. If not, click on the **Select** box to the far right and click **all**.

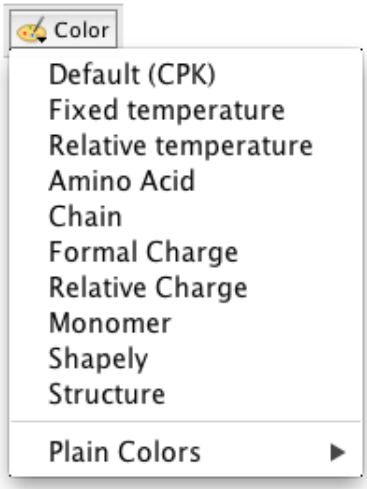


Hidden - hides all atoms
Spacefill - display atoms as spheres the size of their van der Waals radii
Ball and stick - default view showing bonds and atoms
Wireframe - shows the bonds between atoms
Sticks - similar to wireframe but with thicker bonds.
Backbone - displays just the bonds of the αC carbon backbone
Cartoon - follows the backbone but displays secondary structure elements
Ribbons - similar to cartoon but as simple flat ribbons
Rockets - replaces secondary structure with arrows
Strands - like ribbons but not filled in
Trace - follows the trace of the backbone without exactly matching the location of the atoms
Dots - similar to spacefill but with dots rather than solid surfaces
Mesh ribbon - similar to strands but more filled

Try these styles out for yourself.

Colors

Another useful feature is the ability to color structures.



Default (CPK) - industry standard scheme representing atoms by specific colors. See http://en.wikipedia.org/wiki/CPK_coloring
Fixed temperature - according to the temperature factor, a measure of the mobility or uncertainty of a given atom's position referred to an absolute scale of 0-100.
Relative temperature - relative to the lowest and highest temperature factor values
Amino Acid - colored according to traditional amino acid properties
Chain - assigns each macromolecular chain a unique color.
Formal Charge - colors atoms based on their formal charge, or ionic state
Relative Charge - colors atoms based on their partial charge, or electron density
Monomer - a reverse rainbow gradient used to color according to position along a chain starting with blue and finishing with red
Shapely - colored according to the 'Shapely' coloring scheme
Structure - Uses six different colors to distinguish four types of protein secondary structures and DNA vs. RNA.
Plain Colors - simple colors that affect all selected atoms

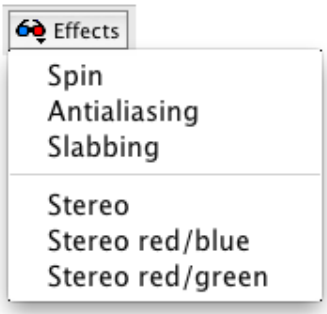
Try combinations of styles and colors.

Select **Backbone** and **Monomer**. This is effective at showing the start and stop of the polypeptide chain. Combine this coloring with rocking the structure and you will have a good idea of how the linear primary sequence folds into the final tertiary structure.

Select **Cartoon** and **Structure**. Now you can clearly see the position of the secondary structure elements. You can see the α-helices and how the β-strands combine to form β-sheets.

Effects

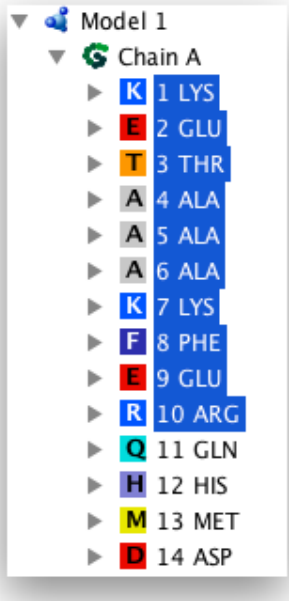
Geneious provides some effects to improve the appearance of your structure or make it easier to see features.



Spin - automatically rotates the structure in the window
Antialiasing - smooth out rough edges making images more suitable for publication
Slabbing - slice the front off a molecule so you can see the interior
Stereo - cross-eye stereo giving a full color image in true 3D
Stereo red/blue - uses traditional red/blue filter glasses to produce a 3D image
Stereo red/green - Alternatively uses green filter for same effect

Selecting parts of the structure

Geneious provides a convenient way to select parts of the structure. This is useful to allow you to highlight features or regions of the structure. At the bottom of the selection window are two buttons labelled **Expand All** and **Collapse All**. Clicking **Expand All** will expand the selection tree to its maximum allowing you to select individual atoms. Click the **Expand All** button and scroll down the window. Notice how the tree expands to show the chain, residues and atoms. If you click the **Highlight** button, any selected region will have a yellow halo. For example, select the first residue (**1 LYS**) with highlighting selected and you will easily be able to see where this Lysine residue is. Similarly, selecting **5 CB** within that residue will highlight just the β-carbon atom.



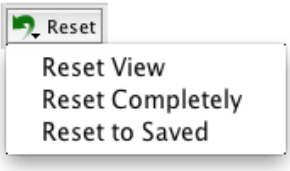
You can also expand and contract parts of the tree by clicking on the arrow to the left of the element you are looking at. Collapse the whole tree by clicking **Collapse All**. Now click the arrow next to **Chain A** and note that it expands out to the next level. You can now easily select individual residues without worrying about the atoms that make them up.

You can also select multiple residues by holding the ^**CTRL** key on Windows/Linux or ⌘**Command** key on a Mac. Now if you click several residues, each will be selected and you can keep adding to the selection in this way. You can also select a range by holding down the ⇧**Shift** key. Hold ⇧**Shift**, click **1 LYS** and **10 ARG** and all residues in between will automatically be selected.

The **Select** button is also provided with a range of groups to select such as inverting the current selection. Click it and select **Inverse** and you should now find that all residues apart from **1 LYS** to **10 ARG** are selected.

Undoing the damage

By now, you have no doubt made many changes to the orientation, style, colors and so on. To return to the original position you can use the reset options.



Reset View - return the structure to it's original orientation
Reset Completely - return view to it's original orientation with default style and color
Reset to Saved - reload the saved state of the molecule

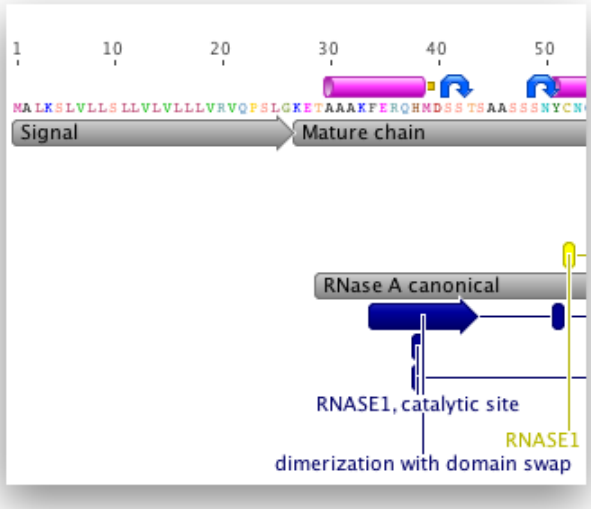
Showing useful information

Now that you are familiar with the molecule viewer it is time to do something useful.

Open the protein sequence **P00656**. Turn on the annotations if necessary and you will see that there are many structural features identified. Make sure you have the **Structure**, **Bond**, **Region** and **Site** annotations checked.

Note that there are secondary structures indicated using type specific cartoons. This protein has a mixture of α-helices, β-strands and turns. These secondary structure annotations have been derived from crystal structures of this protein. Look at the **Text View** for this sequence and you will see references to a number of publications. To find the crystal structures that this sequence correspond to you should click the **View GenBank record on NCBI website** as this provides database cross-references. Under **DBSOURCE** you will see a list of four character **PDB** names including **5RSA** which you have been using already.

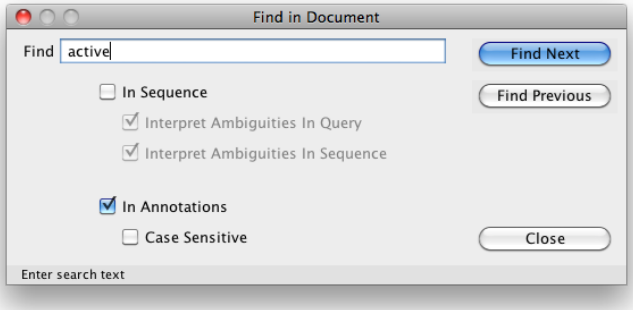
Before returning to the three-dimensional PDB structure, you need to edit the protein sequence you are currently viewing (**P00656**). This is because there is additional sequence which is not present in the crystal data and it will be easier to trace features in the structure if the sequences are identical. Notice that residues 1-26 of P00656 are annotated as **Signal**.



To remove this, click the **Allow Editing** button on the sequence viewer tool bar. Now click on the **Signal** annotation and press the ⌫**Delete** key and choose to remove both the annotation and the sequence itself. Finally, click **Save** to preserve your change.

Highlight features of interest

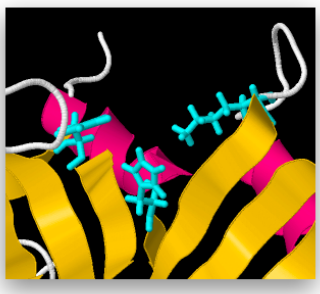
In the annotated protein document there are active site residues. You can find these using Find in Document feature which can be accessed via **Edit→Find in Document**. In the **Find** box, type **active** and select **In Annotations**.



Each time you click **Find Next** Geneious will jump to the next annotation that mentions active. At the bottom of the sequence viewer it will tell you which residue is selected. Note each one and you should find they are **12**, **41** and **119**. These three residues form a catalytic triad that bind divalent cations. Since they are widely spaced in the linear sequence, it will be interesting to highlight their position in the 3D structure to see their actual proximity.

First you need to make the majority of the structure a simple background for the highlighted residues to appear against. Make sure you have selected the whole structure using **Select** and clicking **All**. The **Cartoon** style looks attractive and should suit. Also, color the structure by the **Structure** scheme. Now you can see the helices, sheets and turns. Next, **select residues 12, 41 and 119** in the selection tree by expanding **chain A** out and selecting each while holding the **^CTRL** (Windows/Linux) or **⌘Command** (Mac) keys. When you have selected these three residues, select the **Sticks** style. This will only affect the selected residues. Finally, color them using **plain colors**. **Cyan** stands out well so use that.

You should now see where these three active site residues are and note that they are all directed towards the centre of the RNA binding cleft. More information on Ribonuclease A can be found at: http://en.wikipedia.org/wiki/Ribonuclease_A



When you have finished setting up your molecule, you can click the **Save** button and the state will be preserved for the next time you open the document.

Advanced options

You may have noticed the **Enter command** box. For users familiar with Jmol, you can enter commands directly into here to access functions which are not directly accessible via the Geneious GUI or which can be performed more efficiently via the commands if you know them. More information on available commands can be found at: http://chemapps.stolaf.edu/jmol/docs

Congratulations, you have completed this tutorial.