

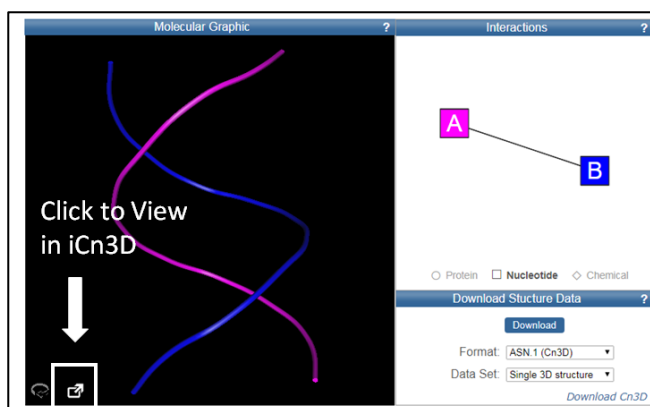
# Instructions for Seeing Molecules in 3D

**Aim:** To understand that protein structure and function, using the bioinformatics tool iCn3D to visualize molecules.

## PART I: Viewing DNA Structure

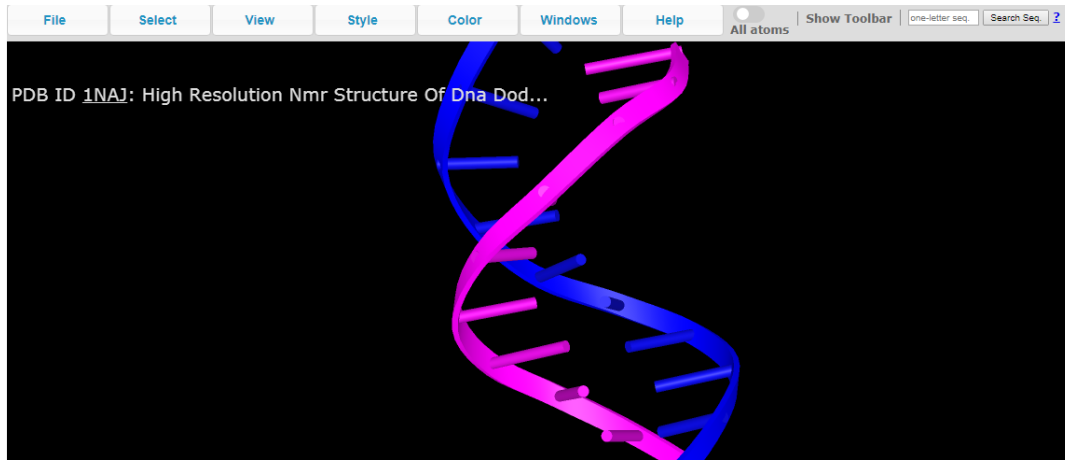
1. Go to the NCBI Structure database: <http://www.ncbi.nlm.nih.gov/structure>.
2. The center top of the page has an open area for search terms. Type **1NAJ** into the search area and click the **Go** button.

We will begin our investigation of 3D structure with a look at a molecule you are already familiar with: DNA. “1NAJ” is the **Protein Databank or PDB ID** for a file that contains structure information for a small piece of double-stranded DNA. A PDB ID is like a catalog number or bar code; it bears no resemblance to the product itself, but allows you to access information in the vast databases at the NCBI.



3. The 1NAJ record will appear as the only result from this search. You are on the **Structure Summary page**, which includes a brief description of the source of the structure. Look at the DNA image that looks like two colored strings winding loosely around each other with a black background. **Click the “View in iCn3D” icon in the lower left corner.**

4. The 1NAJ structure will open in your web browser in the program **iCn3D**. The default visualization is a **Cartoon** format or “rendering” in which only the backbone of the molecule is shown. Each piece (strand of DNA) is shown in a different color.



5. At the top of the page, you will see multiple menus: File, Select, View, Style, Color, Windows and Help.
6. From the **Style** menu, select **Nucleotides** and then **Ball and Stick**. Now you can see each atom in the structure.
7. From the **Color** menu, select **Atom**. Now you can see each atom in the structure. Carbons are black or gray, phosphorus is yellow, oxygen is red, and nitrogen is blue. The hydrogens are not shown.
8. View the DNA in the Structure Window. Drag the structure with your mouse to turn it around. Experiment with controlling the movement and viewing the molecule from multiple angles.
9. Zoom in and out by using the **Z** or **X** keys.
10. Look at the DNA from all sides. **Can you see the double helix?**



11. Open the **View** menu and choose **Auto-Rotation**, and then **Rotate Left**. **What happens?**



Position the DNA molecule the way you want it before proceeding.

12. From the **Style** menu select **Nucleotides**. There are a variety of rendering options available to view the DNA molecule. Explore each way to view the DNA molecule.

Imagine you are teaching a class about DNA. **What viewing option(s) would you use to teach students about DNA structure? Explain the reasons for your decision.**



13. From the **Color** menu, explore the different ways to view the DNA molecule by color. **Select at least three and describe what they do below:**



14. After exploring a few combinations using different viewing and coloring options, which coloring option do you find most useful? Why?



## PART II: Viewing the BRCA1 Protein

Now that you are familiar with iCn3D, we will view part of the BRCA1 protein and part of a second protein that BRCA1 interacts with.

15. Return to the NCBI Structure Page: <http://www.ncbi.nih.gov/structure>
16. Enter the PDB ID for the human BRCA1 protein, **1Y98**, in the Search box and click “Go.”
17. Click to view the structure in iCn3D in your web browser.
18. Use your mouse to view the protein from multiple angles. The default view is the **Ribbon** view. You will see a number of arrows, each pointing different directions. These arrows represent **beta sheets**, one of two common ways that amino acids are organized to form proteins. You will also see a number of helices that look like cork screws. These are **alpha helices**, the second common way that amino acids are organized to form proteins. The blue ribbon is part of the CTIP protein, a protein that BRCA1 needs to interact with in order to repair double-stranded breaks in DNA.
19. From the **Style** menu, select **Proteins** and then **Cylinder and Plate**. With this type of rendering, the beta sheets are still shown as arrows, but the alpha helices are shown as tubes or cylinders.
20. Both of the renderings described above show the **backbone** of the protein. From the **Style** menu, select **Side Chains** and then select **Ball and Stick**. Now the amino chains or **R-groups** are also shown.
21. Go to the **Color** menu and select **Spectrum**. This is also sometimes called the **Rainbow** coloring, in which the beginning, or **N-terminus** of the protein is ‘at the beginning of the rainbow’ (i.e., red/orange) and the end, or **C-terminus** of the protein is ‘at the end of the rainbow’ (i.e., dark blue).
22. When proteins fold into their final 3D shape, the **hydrophobic** (“water fearing”) amino acids tend to fold into the ‘inside’ of the protein, away from the water-rich aqueous environment in the cell, while the **hydrophilic** (“water loving”) amino acids are on the ‘outside’ of the protein, interacting with the water.
23. From the **Color** menu, select **Hydrophobic**. Now all of the hydrophobic amino acids are shown in green, while the hydrophilic amino acids are shown in gray. You can see that the hydrophobic amino acids tend to be folded into the interior of the protein.
24. To see this more clearly, go to the **Style** menu and select **Side Chains** and then **Sphere**. This shows the electron clouds (not drawn to scale) of the side chains of the amino acids. Again you can see that the hydrophobic amino acids are found in the interior of the protein and the hydrophilic amino acids are found on the exterior of the protein.
25. Continue to explore different ways to view the protein structure, as you did with the DNA structure in Part I.

26. Select your favorite way to view the protein. Which is your favorite **rendering**, or 'way to view the structure,' and why?



27. In the space below, describe or draw the structure of the BRCA1 protein.



28. To see another way to view amino acid chemistry, return to the **Color** menu and select **Charge**. Now the negative (acidic) amino acids are colored **red** while the positive (basic) amino acids are colored **blue**.
29. To see which amino acids are which, from the **Sequences and Annotations** menu on the right side of the screen, click the **Details** tab. If the **Sequences and Annotations** menu is not displayed, from the **Windows** tab in the top menu select **View Sequences and Annotations**. [See the image below as an example.]

The screenshot shows a protein structure viewer interface. On the left is a 3D ball-and-stick model of a protein structure. On the right is a panel titled 'Sequences and Annotations'. The panel has two tabs: 'Summary' and 'Details'. The 'Details' tab is selected. Under 'Annotations', there are checkboxes for 'All', 'Conserved Domains' (checked), 'ClinVar', 'Functional Sites', 'Custom', '3D Domains', 'SNPs', and 'Interactions'. Below this is a 'Show All Chains' button. A selection bar shows '+ Selection: Name: seq\_1' with 'Save' and 'Clear' buttons. The 'Proteins:' section lists 'Annotations of 1Y98\_A: BREAST CANCER TYPE 1 SUSCEPTIBILITY PROTEIN' with an 'Add Track' button. Below this is a sequence alignment showing NCBI Residue Numbers (1 to 1680) and PDB Residue Numbers (1650 to 1680). The sequence is: 1 v n k R M S M V V S G L T P E E F M L V Y K F A R K H H I T L T N L I. The domain 'BRCT' is highlighted in yellow, spanning residues 85 to 74. The sequence is also shown in a 1D representation with a yellow arrow indicating the domain. Below this is another entry: 'Annotations of 1Y98\_B: CTIP PHOSPHORYLATED PEPTIDE' with an 'Add Track' button.

30. Note that in the Details section of the Sequences and Annotations window, you can see the BRCA1 protein sequence (shown by the one-letter amino acid abbreviations). The amino acid letters are color-coded the same way that the protein structure is color coded. For example, the Glutamic Acid amino acids (abbreviated "E") are red, because they are acidic. The amino acids that comprise the beta sheets (gold arrows) and the alpha helices (red squiggles) are also shown.
31. Select an amino acid in the BRCA1 protein sequence by clicking on it. What do you observe as a result in the protein structure?



32. Below the amino acid sequences are numbers that correspond to the location of each amino acid within the protein structure. For example, the first amino acid in this protein structure is a Methionine (M) and it is amino acid number 1650 within the total BRCA1 protein. [Remember that this protein structure is only a portion of the entire BRCA1 protein].
33. Go to amino acid M1775. This is the amino acid that can mutate to Arginine (R), noted by scientists as "M1775R," which leads to a dramatic increase in one's risk of cancer. This is the most common cancer-causing mutation in BRCA1.
34. Where is M1775 located on the protein? Is it exposed at all to the surface of the protein? If it is, then it could interact with other proteins.



35. Why do you think that this mutation may increase one's risk of cancer?