

Molecular Modeling of Ras


The G-protein Ras has been thoroughly studied, because of the important role that Ras plays in cell division. In both normal and cancer cells, Ras plays a central role in signal transduction processes that lead to cell division. Furthermore, scientists estimate that ~30% of human tumors carry mutant alleles of Ras. The purpose of this laboratory is to help you review what you have learned about protein structure while studying the important signal transduction protein Ras.


Begin by using your class notes and/or assigned reading to answer Question 1 on the worksheet at the end of this document.

Next, obtain and open a file containing the Ras protein structure.

- Double click the Spartan '06 icon on your computer's desktop.
- Click on the **Search** menu at the top of the page and then select **Databases**.
- Click on the Protein Data Bank (**PDB**) tab and type 1aa9 in the **By PDB ID** box at the bottom right of the window that pops up.
- Click on the **Search** button. Click **OK** in the window indicating that the search is complete.
- After Spartan provides list of matches/hits (there should only be one hit), click on *Iaa9* to highlight it and then click on the **Retrieve** button.
- Close the PDB dialog box by clicking on the "X" in the upper right-hand corner of this small window.

Now let's take a close look at Ras.

- Begin by reviewing how to rotate structures and how to zoom in and out. Left click, hold, and drag the mouse on the main molecule screen to rotate the molecule in three dimensions. Right click, hold, and drag the mouse to move the molecule to a different location on the screen. Right click, hold, and drag the mouse while holding down the shift key to zoom in and out. Zoom out so that you can see the entire structure of Ras.
- Before studying the protein itself, let's look at what it is bound to.
- The ligand (what Ras is bound to) is shown as a partially transparent, and is in the space-filling form. To take a look at the ligand by itself, we must extract it from the protein, using the following steps:
 - o Click on the **Search** menu at the top of the page. Select **Ligands**.
 - o Click on an atom of the ligand and the message "Ligand GDP180 Selected" should appear at the bottom of the page.
 - o Click on the **Extract Ligands** button.
 - o In the window that pops up, click on (put check marks in) the boxes corresponding to *Ligand Structures*, *Grow Hydrogens*, and *Repair Bonds*. Click **OK**.
 - o Click on part of the protein itself and close the protein structure by clicking on the close button () at the top of the page. Do not save the changes to the protein file.

- Now that you are looking at the ligand by itself, what do you see? Is the ligand an amino acid? a lipid? a nucleotide? Use your observations to answer Questions 2 and 3 on your worksheet.
- Close () the ligand file. Do not save the changes to this document.
- Re-open the protein file by clicking on **Search>Databases**. Select *Iaa9*, click **Retrieve**, and close the PDB dialog box.
- To view the space-filling model of Ras, click on the **Model** menu at the top of the page and de-select (remove the checkmark from) *Ribbons*. Then go back to the **Model** menu and select *Space Filling*. Answer Question 4 on the worksheet.
- Now lets take a look at the secondary structure of Ras by clicking on the **Model** menu at the top of the page and selecting (adding a checkmark to) *Ribbons*. Then go back to the **Model** menu and select *Hide* in the top portion of the menu. The ribbon diagram you are now looking at allows you to visualize the structure of the polypeptide backbone, making it easier to see α helices (curlicues) and β sheets (several straight-ish portions of ribbon aligned next to each other to form a flat sheet). Rotate this structure to look at it from several different angles, and use your observations to answer Questions 5 and 6.

Finally, let's consider what happens when Ras is mutated. Begin by using your lecture notes and/or the assigned reading to answer Question 7.

One of the most common amino acid residues that is altered in cancer cells is the 12th amino acid, which normally is a glycine. In many cancer cells, the Ras gene has been altered so that the 12th amino acid is now a valine. Use this information and the genetic code table from your class notes to answer Question 8 on the worksheet. Changing the 12th amino acid of Ras from glycine to valine impairs Ras' ability to hydrolyze GTP to GDP, thus this mutant form of Ras cannot be switched off. Given that mutating glycine 12 inhibits that ability of Ras to hydrolyze GTP, where would you expect this amino acid residue to lie within the three dimensional structure of Ras? Near the nucleotide binding site, or somewhere else in the protein? Record your answer on the worksheet (Question 9).

- Now let's test your prediction by visualizing where this 12th amino acid is within the overall structure of Ras. Begin by clicking somewhere on the ribbon representing Ras' polypeptide backbone. Notice that in the lower right hand corner of the screen, a message like "Peptide (GLN129)" will appear. The number tells you which amino acid you are looking at... in the example given, the 129th amino acid in Ras has been highlighted. Use this approach to find the 12th amino acid, which should be a glycine. When you've got it, the message "Peptide(GLY12)" will appear in the bottom right-hand corner of the screen.
- Once you've selected the glycine at position 12, go up to the **Display** window and click on **Properties**.
- Click on the downward pointing arrow at the bottom right of this window. Then use the **Model** menu to change from *<ambient>* to *Space Filling*. Use the information you obtain to answer Question 10 on the worksheet.

Name: _____

Ras Molecular Modeling Worksheet

1. What role does Ras play in the transduction of growth signals? Is Ras active and when it is bound to GTP or GDP? In your explanation be sure to describe which protein activates Ras and which protein is a downstream target of Ras.
2. What molecule is bound to the Ras protein you are analyzing, GDP or GTP? Is it a nucleotide, an amino acid, or a fatty acid? How do you know? In your answer, be sure to describe the structure of this ligand. What smaller components is it made of?
3. Based on the structure of the ligand you extracted, is the Ras protein you are studying active or inactive?
4. Based on the space-filling view of the Ras structure, how would you describe the Ras protein? Is it globular or filamentous? Does it look solid and tightly packed or loose and “airy”?
5. What type of non-covalent bond stabilizes secondary structures in proteins? Are such bonds between amino acid side chains or between atoms in the polypeptide backbone?
6. How many alpha helices do you see?

There is a beta sheet running through the middle of the protein. How many stretches of protein are part of this beta sheet?
7. The structure you have been working with corresponds to the Ras protein which is encoded by the Ras proto-oncogene. What is the difference between a proto-oncogene and an oncogene?
8. Wild-type (non-cancer-associated) alleles of Ras encode glycine as the 12th amino acid. What are the four possible codons for this amino acid?

Many cancer-associated alleles of Ras encode valine as the 12th amino acid. What are the four possible codons for valine?
9. Given that mutating glycine 12 inhibits that ability of Ras to hydrolyze GTP, where would you expect this amino acid residue to lie within the three dimensional structure of Ras? Near the nucleotide binding site, or somewhere else in the protein? Briefly explain your answer.
10. After changing glycine 12 to a space-filling model, describe the location of this critical amino acid residue.

Was the prediction you made in Question 9 correct?