

## Ras and Cell Signaling Exercise

### Learning Objectives

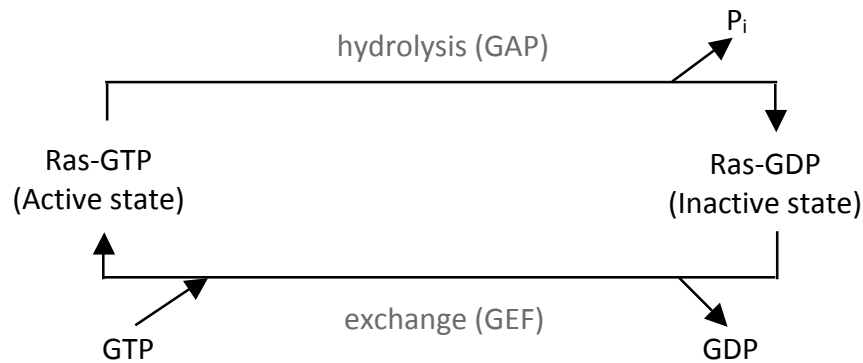
In this exercise, you will use StarBiochem, a protein 3D- viewer, to explore:

- the structure of the Ras protein
- the active and inactive state of Ras and the amino acids that contribute to these two states

### Background

Ras proteins are plasma membrane bound proteins that communicate signals from the outside of the cell to the nucleus. Ras proteins are essential components of various signal transduction pathways that regulate growth, proliferation, differentiation, and apoptosis. The Ras protein family is divided into five major categories based on their sequence and functional similarities: Ras, Rho, Rac, Ran, and Arf.

Ras proteins function as molecular switches by cycling between an active GTP-bound state and an inactive GDP-bound state. Conversion from the GTP-bound state to the GDP-bound state is mediated by the intrinsic GTPase activity of Ras, which hydrolyzes GTP to GDP, and by the GTPase activating protein (GAP). On its own, Ras protein can hydrolyze GTP, however, this process is too slow for efficient Ras function. Binding of GAP to Ras protein accelerates GTP hydrolysis by Ras. The reverse process, the exchange of GDP to GTP, is facilitated by a guanine nucleotide exchange factor (GEF).

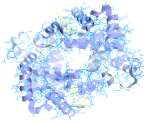


Mutations in Ras genes can result in permanent activation and cause inappropriate signaling even in the absence of activating extracellular signals. Given that activation of Ras signaling results in cell growth and division, dysregulated Ras signaling can ultimately lead to cancer. Mutations in Ras genes are found in 20-25% of all human tumors and are particularly common in specific tumor types such as leukemias and colon cancer.

### Getting started with StarBiochem

To help you learn how to use the program, a StarBiochem User Guide is available online.

- To begin using StarBiochem, please navigate to <http://web.mit.edu/star/biochem>.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking you if you trust the certificate.
- In the top menu under File click on **Open/Import**, select "5P21" and click **Open**.



StarBiochem

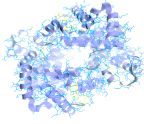
Name \_\_\_\_\_

You are now viewing the structure of a fragment of the Ras protein (5P21) with each bond in the protein drawn as a line (“bonds only” view).

Practice changing the viewpoint of this protein in the view window:

	<b>Mac</b>	<b>PC</b>
<b>TO ROTATE</b>	<b>click</b> and <b>drag</b> the mouse	<b>left-click</b> and <b>drag</b> the mouse
<b>TO MOVE UP/DOWN RIGHT/LEFT</b>	<b>apple-click</b> and <b>drag</b> the mouse	<b>right-click</b> and <b>drag</b> the mouse
<b>TO ZOOM</b>	<b>option-click</b> and <b>drag</b> the mouse	<b>Alt-left-click</b> and <b>drag</b> the mouse

Take a moment to look at the “bond only” view of Ras (5P21). Before proceeding to answer the questions, you can review the basic structures and terms on the next page which you can refer to during this exercise.



## PROTEIN STRUCTURE BASICS

Each protein has the following three levels of protein structure:

### Primary structure

Lists the amino acids that make up a protein's sequence, but does not describe its shape.

### Secondary structure

Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

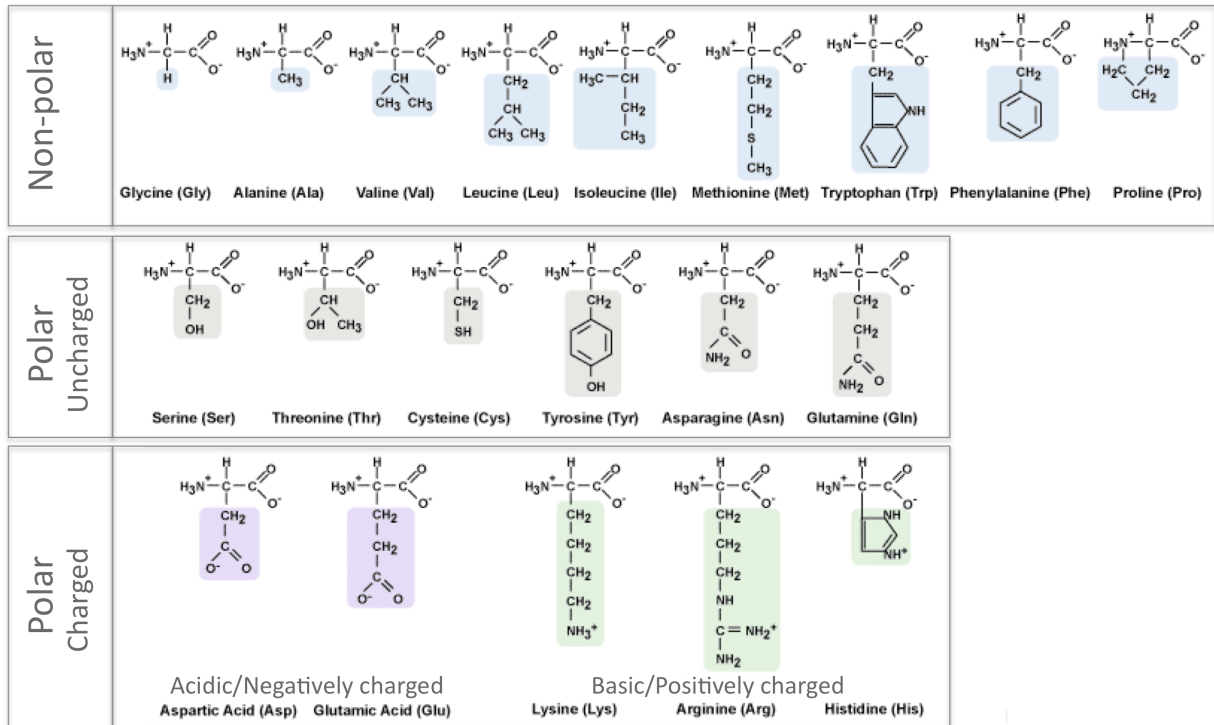
### Tertiary structure

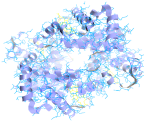
Describes the entire folded shape of a whole protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. How these proteins interact and fold to form a larger protein complex is termed **Quaternary structure**.

## CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different 'R' groups, highlighted in various colors below.





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Name \_\_\_\_\_

**1 How would you classify this protein: monomeric or oligomeric?**

- To distinguish between the polypeptide chains that can make up a protein, under **Structure** click on **Quaternary**.
- Click on **Chain**.
- Each polypeptide chain/monomer is highlighted by a different color.

Answer

**2 Based on the primary sequence of Ras (5P21), what is the approximate length of the mature Ras mRNA?**

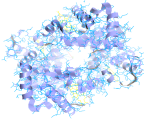
*Hint: Membrane proteins have an additional amino acid sequence called the signal recognition peptide, comprised of 15-20 amino acid residues, that targets them to the endoplasmic reticulum. The signal recognition peptide sequence is found within the mature mRNA, but is cleaved after the protein is inserted into the ER lumen and therefore is not found in the mature protein. Remember that each amino acid is specified by a three nucleotide base codon.*

Answer

**3 Identify and draw the other structural element shown in this structure.**

- Within the main menu go to **View**.
- Click on **Reset molecule**.
- Under **Selection controls** click on **Atom**.
- Close the selection control window.
- Under **PDB tree**, click on **5P21** and then click on all the heteroatoms present by simultaneously holding **Control** and **Apple** key(Mac)/**right-click** (PC).
- In the **View Controls** panel, set the **Unselected** transparency slider to “**0.1**” keeping the **Selected** transparency slider at “**1**”.
- Click on **Draw** within the **Atoms** box to see what atoms are present. Each atom is color coded: Carbon is grey, Nitrogen is blue, Oxygen is red and in this structure, Phosphorous is orange and Magnesium is green.

Answer



**4 What form of Ras is shown in this structure: active or inactive? Explain why.**

Answer

**5** We will now analyze the secondary structure of Ras (5P21).

- Within the main menu go to **View**.
- Click on **Reset molecule**.
- Under **Structure**, click on **Secondary**.
- Explore the different secondary structures by individually clicking **Helices**, **Sheets** or **Coils**.
- Alternatively, you can click on **All ribbons** within the **Show Ribbons** box to view all secondary structures simultaneously.

**a) Which of the following options best describes Ras' secondary structure? Circle your choice.**

Answer

- Beta sheets surrounded by helices and coils.
- Coils surrounded by beta sheets and helices.
- Helices surrounded by sheets and coils.

We will now explore one of the secondary structures within Ras.

- Within the main menu go to **View**.
- Click on **Reset molecule**.
- Under **Structure**, click on **Secondary**.
- Under **Secondary**, click on "#5" within the **Helix selection** window.

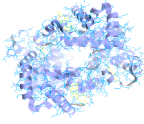
**b) Is helix 5 located at the N- or the C-terminus of the protein? Explain.**

Answer

**c) How many amino acid residues does helix 5 have?**

*Hint: look at the amino acids that are highlighted in **Primary** under **Structure**.*

Answer



d) The C-terminus of Ras is involved in targeting Ras to the plasma membrane. **Given the function of the C-terminus, what type of amino acids would you expect to find in this region?** Your choices are 'hydrophobic', 'acidic', 'basic', or 'polar'. Explain.

Answer

4 Ras is comprised of five major 'G' motifs that bind to GDP/GTP directly. These motifs are: G1 (amino acids #10-17), G2 (amino acid #35), G3 (amino acids #57-60), G4 (amino acids #116-119) and G5 (amino acids #145-147).

- Within the main menu go to **View**.
- Click on **Reset molecule**.
- Under **Structure**, click on **Primary**.
- Highlight the amino acids within a motif by individually clicking on them and simultaneously pressing **Control** and **Apple** (Mac)/**right-click** (PC).
- Under **Selection Controls**, click on **Atoms**.
- Under **PDB tree** click on **5P21**.
- Click on "GNP". Every time you click on a new set of amino acids within **Primary**, you have to re-select "GNP" within **PDB tree**.

a) Highlight the amino acid residues within the G1 and G2 motifs. **To which part of GTP/GDP do these motifs bind?** Your choices are 'guanine base', 'sugar' or 'phosphates'.

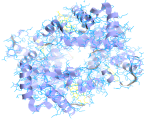
- Within **View Controls**, set the **Unselected** transparency slider to "0.7" keeping the **Selected** transparency slider at "1".
- Click on **Draw** within the **Atoms** box to see what atoms are present. Each atom is color coded: Carbon is grey, Nitrogen is blue, Oxygen is red, Phosphorous is orange and Magnesium is green.

Answer

b) **Which of these two G motifs can bind to both GTP and GDP?** Explain your choice.

Answer

c) **Which of these two G motifs can bind only to GTP? State the most likely mode of interaction between this motif and GTP.** Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'. **Draw this interaction and indicate the residues involved.** Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.



Answer

d) Highlight the amino acid residues within the G3, G4 and G5 motifs. **Which motif(s) interact with the guanine base of GTP/GDP?**

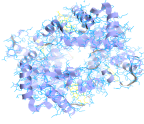
Answer

e) **Identify the amino acids within the G3, G4 and G5 motifs that interact with the guanine base of GTP/GDP by hydrogen bonding. Draw this interaction(s) and indicate the residues involved.** Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.

Answer

g) Amino acid #30 interacts with the sugar component of GTP/GDP. **What is the most likely mode of interaction between these two?** Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'. **Draw this interaction and indicate the residues involved.** Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.

Answer



**h) The interaction of Ras with GTP/GDP also involves  $Mg^{++}$  ions. *Identify the part of GTP/GDP that interacts with  $Mg^{++}$  ions.* Your choices are 'guanine base', 'sugar' or 'phosphates'. *Draw this interaction.***

Answer

**j) *State the mode of interaction between  $Mg^{++}$  ions and GTP/GDP.*** Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'.

Answer

**5** Mutations in the genes that encode Ras protein family members are very common in all human tumors. Therefore it is reasonable to speculate that a pharmacological approach that inhibits Ras activity may represent a possible method to treat certain types of cancer.

- Within the main menu go to **View**.
- Click on **Reset molecule**.

Here are three individual amino acid substitutions that may affect Ras activity.

- Substitution 1: amino acid residue #17 substituted by asparagine.
- Substitution 2: amino acid residue #119 substituted by asparagine.
- Substitution 3: amino acid residue #61 substituted by lysine.

For each of these amino acid substitutions:

**a) *Identify the original amino acid present at these locations.***

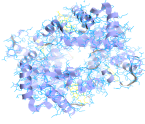
Answer

**b) *State which of these substitutions may result in a Ras protein that is active at all times.***

Explain your choice.

Answer





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**c) State which of these substitutions may result in a Ras protein that is inactive at all times.**

Explain your choice.

Answer

**Keywords:**

Ras proteins, GTP, GDP, GEF, GTPase, cancer, tumors, differentiation, apoptosis, nucleotides, sugars, and endoplasmic reticulum.

**Thought questions**

**1** A change in the activity of Ras can influence various cellular processes such as proliferation, differentiation and cell death. Propose a mechanism to explain how a single protein can influence diverse cellular processes.

**2** Mutations in Ras protein are commonly observed in most cancers and are regarded as 'gain of function' mutations. Would you suspect these mutations are dominant or recessive? Explain.