

Course Materials for Week 3: G-Proteins

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Assignment Questions (Part 1)

1. Why are Switch I and Switch II important in the mechanism of the G-protein molecular switch?
 - a. Because they undergo structural change when GTP is hydrolyzed
 - b. Because they bind the gamma phosphate of GTP
 - c. Because they bind the alpha phosphate of GTP
 - d. Because they undergo structural change when GDP is exchanged with GTP
 - e. None of the above

2. How do other proteins modify the basic cycle of GTP-binding proteins? The slow dissociation of GDP is accelerated by _____, the slow GTPase reaction by _____.
 - a. GEFs, GDP hydrolysis
 - b. GAPs, GEFs
 - c. GEFs, GAPs
 - d. GAPs, GTP hydrolysis
 - e. None of the above

3. What molecular feature of GTP is involved in the GTP-binding protein switch (select all that apply)?
 - a. alpha phosphate
 - b. beta phosphate
 - c. gamma phosphate
 - d. None of the above

4. What are the important biochemical aspects of G proteins that allow for it to be regulated (select all that apply)?
 - a. Slow dissociation of nucleotide
 - b. Slow GTPase activity
 - c. Affinity for nucleotide in the mM range
 - d. Mg²⁺ dependent affinity and GTPase activity

- e. None of the above
5. Which alterations of the k_{diss} and k_{off} parameters will increase the active form of a GTP binding protein (select all that apply)? (5 pts)
- a. Increasing k_{diss}
 - b. Increasing k_{off}
 - c. Decreasing k_{diss}
 - d. Decreasing k_{off}
 - e. None of the above

Assignment questions (Part 2)

6. The following amino acid(s) are important for making the gamma-phosphate more susceptible to a nucleophilic attack by water (select all that apply):
- a. Glutamine
 - b. Glycine
 - c. Arginine
 - d. Asparagine
 - e. None of the above
7. Which of the following lead to Ras being an oncogene (select all that apply)?
- a. Loss of upstream activation
 - b. Inability of GAPs to hydrolyze GTP
 - c. Gly12 is mutated into a different amino acid
 - d. Ras cannot be turned off
 - e. Gln61 is mutated into a different amino acid
 - f. None of the above
8. What are possible ways of treating a cancer that has constitutively active Ras (select all that apply)? (5 pts)
- a. Block hormones from activating the Ras pathway
 - b. Block Ras-GTP from interacting with GEFs
 - c. Synthesize a small molecule that can hydrolyze GTP from Ras-GTP
 - d. Block Ras-GTP from interacting with downstream effectors
 - e. None of the above

9. Why do G proteins become loaded with GTP once GDP is removed (select all that apply)? (5 pts)

- a. The reaction is energetically favorable
- b. There is significantly more GTP than GDP in the cell
- c. The reaction is energetically unfavorable
- d. There is significantly more GDP than GTP in the cell
- e. None of the above

10. The following amino acid(s) is important for positioning the water molecule that is necessary for the GTP hydrolysis reaction (select all that apply): (5 pts)

- a. Glutamine
- b. Glycine
- c. Arginine
- d. Asparagine
- e. None of the above

11. What part of the GTP hydrolysis reaction does the GAP catalyze (select all that apply)? (4 pts)

- a. Isomerization of Ras-GTP to make GTP hydrolysis more favorable
- b. Cleavage of GTP into GDP and Pi
- c. Pi release from Ras-GDP
- d. None of the above

12. In RapGAP, the amino acid necessary for RapGTP hydrolysis is (select all that apply): (5 pts)

- a. Glutamine
- b. Glycine
- c. Arginine
- d. Asparagine
- e. None of the above

13. The amino acid(s) that, when mutated, cause steric interference in the active site of the GTP hydrolysis reaction are (select all that apply): (5 pts)

- a. Glutamine

- b. Glycine
- c. Arginine
- d. Asparagine
- e. None of the above

14. The conversion of Ran-GTP to Ran-GDP occurs in sequential transition steps, which are (select all that apply): (5 pts)

- a. Binding of the GAP followed by hydrolysis of GTP
- b. Binding of the GAP followed by nucleophilic attack by water
- c. Hydrolysis of GTP followed by release of the GAP
- d. Hydrolysis of GTP followed by release of Pi
- e. None of the above

15. This amino acid is an important residue in the RasGAP for mediating GTP hydrolysis: (5 pts)

- a. Glutamine
- b. Glycine
- c. Arginine
- d. Asparagine
- e. None of the above

16. In class this week, we will be discussing the following question:

Suppose scientists discovered a new Ras-like protein, Arr. They found that mutations in Arr and/or ArrGAP were implicated in a form of cancer. Suggest two possible mechanisms by which a mutation in either Arr or ArrGAP could result in cancer. For each:

- a. Describe the mutation
- b. Explain how the mutation would affect the G-protein cycle
- c. Please provide an answer to each of the sub-questions using full sentences and clear statements based on scientific principles (10 pts).

Assignment Answers

1. a, b, and d
2. c
3. c
4. a, b, and d
5. a and d
6. a and c
7. a, b, c, d, and e
8. c and d
9. b
10. a
11. b
12. d
13. b
14. d
15. c
16. The grading rubric for this question is included below:

Assignment (10pts)	Pass (points)	No Pass (no point)
For each mechanism: Length and clarity (2 pts per mechanism, 4 pts total)	The student described the mutation and provided a clear explanation of the G protein cycle.	The student did not describe the mutation and did not provide a clear explanation of the G protein cycle.
For each question: Content of answer (3 pts per question, 6 pts total)	The answer relates directly to the question and is based on valid scientific data, models or principles.	The answer is off topic and it is not based on any scientific data, models or principles.

In-Class Quiz Question

1. The conversion of Ran-GTP to its inactive state follows which reaction scheme (select all that apply): (5 pts)

- a. $\text{Ran-GTP} \rightarrow \text{Ran-GDP.Pi} \rightarrow \text{Ran-Pi} + \text{GDP}$
- b. $\text{Ran-GTP} \rightarrow \text{Ran-GDP} \rightarrow \text{Ran-GDP.Pi}$
- c. $\text{Ran-GTP} \rightarrow \text{Ran-Pi} \rightarrow \text{Ran-GDP} + \text{Pi}$
- d. $\text{Ran-GTP} \rightarrow \text{Ran-GDP.Pi} \rightarrow \text{Ran-GDP} + \text{Pi}$
- e. None of the above

2. Which step in this reaction is the rate-limiting step? (5 pts)

- a. $\text{Ran-GTP} \rightarrow \text{Ran-GDP.Pi}$
- b. $\text{Ran-GDP.Pi} \rightarrow \text{Ran-Pi}$
- c. $\text{Ran-GDP.Pi} \rightarrow \text{Ran-GDP} + \text{Pi}$
- d. $\text{Ran-GTP} \rightarrow \text{Ran-GDP}$
- e. None of the above

3. Imagine you are designing a drug targeting a mutation in Arr or in ArrGAP, which is known to cause a form of cancer.

Suggest a possible amino acid drug target and the mechanism by which the drug could affect the G protein cycle of the mutated protein (10 pts).

In-Class Quiz Answer

- 1. d
- 2. c
- 3. There are many answers to this question. The most important thing is that they clearly understand the reaction cycle of the G-protein.

Here are answers that should get credit if they are accompanied by good explanations:

- Designing a drug that inhibits the function of Ras (e.g. inhibit post-translational modifications such as farnsylation which help Ras function)
- Inhibit downstream effectors in the Ras pathway to eliminate that pathway from being constitutively activated.
- Determine what the mutation in Arr/ArrGAP is and design a drug to act in the active site of GTP hydrolysis. For example, the drug could properly position the water or the gamma-phosphate or neutralize charges on the gamma-phosphate depending on what the cancer-causing mutation is
- If the mutation is in the ArrGAP protein, then design a drug mimicking the Arginine finger or Asn finger (e.g. such as found in RapGAP) so the GTP can be hydrolyzed

Rubric

In Class (10pts)	Pass (points)	No Pass (no point)
Completeness and clarity (5 pts total)	The student described the target and provided a clear explanation of the mechanism.	The student did not describe the mutation and did not provide a clear explanation of the mechanism.
Content of answer (5 pts total)	The answer relates directly to the discussion question and includes one of the accurate answers provided in the key, or is based on valid scientific data, models or principles discussed in class or in the talk.	The answer is off topic and it is inaccurate and cannot be justified scientifically.