Jeopardy

Regulation  Mixed Bag  Carbohydrates  Proteases  Signaling

$100  $100  $100  $100  $100

$200  $200  $200  $200  $200

$300  $300  $300  $300  $300

$400  $400  $400  $400  $400

$500  $500  $500  $500  $500

Final Jeopardy
Regulation - $100

- What reaction does phosphatase catalyze?
- Bonus: What reaction does kinase catalyze?
What reaction does phosphatase catalyze?

Bonus: What reaction does kinase catalyze?

Phosphatases are enzymes that catalyze the removal of phosphoryl groups attached to proteins.
What reaction does phosphatase catalyze?

Bonus: What reaction does kinase catalyze?

Phosphatases are enzymes that catalyze the removal of phosphoryl groups attached to proteins.

Bonus: kinases catalyze the addition of phosphoryl groups.
What reaction does phosphatase catalyze?

Bonus: What reaction does kinase catalyze?

Phosphatases are enzymes that catalyze the removal of phosphoryl groups attached to proteins.

Bonus: kinases catalyze the addition of phosphoryl groups.

![Diagram of phosphatase reaction](image)
This hormone is generated during exercise or in a “fight or flight” situation.

Bonus: What type of receptor does this hormone interact with?
This hormone is generated during exercise or in a “fight or flight” situation.

Bonus: What type of receptor does this hormone interact with?

Epinephrine or adrenaline
Regulation - $200

- This hormone is generated during exercise or in a “fight or flight” situation.

- Bonus: What type of receptor does this hormone interact with?

- Epinephrine or adrenaline
- $\beta$-adrenergic receptor, a 7 Transmembrane receptor
Define feedback inhibition and give an example of feedback inhibition.
Define feedback inhibition and give an example of feedback inhibition.

- Feedback inhibition: inhibition of an enzyme by its product or a later product in its pathway (ex. The final product of a long pathway inhibits the first enzyme in the pathway)
- CTP, the end product of pyrimidine biosynthesis, inhibits ATCase.
How is protein kinase A regulated?

Bonus: what is one pathway in which protein kinase A is involved?
How is protein kinase A regulated?

Bonus: what is one pathway in which protein kinase A is involved?

Inactive form: R2C2 dimer
How is protein kinase A regulated?

Bonus: what is one pathway in which protein kinase A is involved?

- Inactive form: R2C2 dimer
- R = regulatory, C = catalytic
How is protein kinase A regulated?

- Bonus: what is one pathway in which protein kinase A is involved?

- Inactive form: R2C2 dimer
- R = regulatory, C = catalytic
- Active form: each R subunit binds 2 cAMP, dissociates as R2 dimer, releasing the 2 C domains to do their chemistry
How is protein kinase A regulated?

Bonus: what is one pathway in which protein kinase A is involved?

- Inactive form: R2C2 dimer
- R = regulatory, C = catalytic
- Active form: each R subunit binds 2 cAMP, dissociates as R2 dimer, releasing the 2 C domains to do their chemistry

Bonus: glycogen breakdown or β-adrenergic receptor signaling
How is protein kinase A regulated?

- Inactive form: R2C2 dimer
- R = regulatory, C = catalytic
- Active form: each R subunit binds 2 cAMP, dissociates as R2 dimer, releasing the 2 C domains to do their chemistry
- Bonus: glycogen breakdown or β-adrenergic receptor signaling

- Bonus: what is one pathway in which protein kinase A is involved?
How does caffeine give you a little bit of a “buzz”? 
Regulation - $500

- How does caffeine give you a little bit of a “buzz”?
  - Phosphodiesterase is inhibited by caffeine
How does caffeine give you a little bit of a “buzz”?  

- Phosphodiesterase is inhibited by caffeine
- Phosphodiesterase is responsible for converting cAMP back to AMP
How does caffeine give you a little bit of a “buzz”?

- Phosphodiesterase is inhibited by caffeine
- Phosphodiesterase is responsible for converting cAMP back to AMP
- Caffeine gives a small boost to blood glucose by keeping cAMP levels higher
If there are 10 times as much ADP and Pi than ATP at equilibrium at 298K in water, what is the $K_{eq}$?
mixed bag - $100

If there are 10 times as much ADP and Pi than ATP at equilibrium at 298K in water, what is the $K_{eq}$?

- $K_{eq} = \frac{[ADP]_{eq}[Pi]_{eq}}{[ATP]_{eq}} = \frac{(10)(10)}{1} = 100$
What reaction does carbonic anhydrase catalyze?

Bonus: What is the limiting step in carbonic anhydrase catalysis?
Mixed Bag - $200

- What reaction does carbonic anhydrase catalyze?
- Bonus: What is the limiting step in carbonic anhydrase catalysis?

- Carbonic anhydrase catalyzes the joining of carbon dioxide and water to form carbonic acid.
- The limiting step in the action of carbonic anhydrase is the abstraction of the proton from water.
Name the two types of subunits in ATCase.

Bonus: How is ATCase allosterically regulated?
Name the two types of subunits in ATCase.

Bonus: How is ATCase allosterically regulated?

Two types of subunits in ATCase: regulatory and catalytic
Name the two types of subunits in ATCase.

Bonus: How is ATCase allosterically regulated?

- Two types of subunits in ATCase: regulatory and catalytic
- CTP binds ATCase, promotes T state, less reactive
Two types of subunits in ATCase: regulatory and catalytic

- CTP binds ATCase, promotes T state, less reactive
- ATP binds ATCase, promotes R state, more reactive
What is the free energy change for coupling the synthesis of ATP to dephosphorylation of G6P?

ATP + H2O $\rightarrow$ ADP + Pi $\Delta G^\circ' = -30.5$ kJ/mol

G6P + H2O $\rightarrow$ glucose + Pi $\Delta G^\circ' = -13.8$ kJ/mol
What is the free energy change for coupling the synthesis of ATP to dephosphorylation of G6P?

ATP + H2O → ADP + Pi  \( \Delta G^\circ' = -30.5 \text{ kJ/mol} \)
G6P + H2O → glucose + Pi  \( \Delta G^\circ' = -13.8 \text{ kJ/mol} \)

Net reaction: ADP + G6P → ATP + glucose
\(-13.8 \text{ kJ/mol} + 30.5 \text{ kJ/mol} = 16.7 \text{ kJ/mol} \)
(this reaction will not proceed spontaneously)
**Mixed Bag - $500**

- Name the three different zymogens discussed in class required for blood clotting. What are the names of their active and inactive forms?
- Bonus: Which of these is inhibited by warfarin?
- Double bonus: What is the very first signal to stimulate the blood clotting process?
Mixed Bag - $500

- Name the three different zymogens discussed in class required for blood clotting. What are the names of their active and inactive forms?
- Bonus: Which of these is inhibited by warfarin?
- Double bonus: What is the very first signal to stimulate the blood clotting process?

- Thrombin: inactive: prothrombin, active: thrombin cleaves fibrinogen to make fibrin
- Fibrin: inactive: fibrinogen, active: fibrin activated by thrombin
- XIIIa aka transglutaminase: inactive: XIII, active: XIIIa activated by thrombin
- Bonus: Vitamin K epoxide reductase is inhibited by warfarin.
- Double bonus: exposure of collagen (connective tissue)
In what conformation are most biological sugars found?
In what conformation are most biological sugars found?

- D-conformation
Carbohydrates - $200
Carbohydrates - $200

- Draw the straight chain, furanose, and pyranose forms of galactose.
- Bonus: Identify if your drawings are D or L and α or β.
Draw the straight chain, furanose, and pyranose forms of galactose.

Bonus: Identify if your drawings are D or L and α or β.
Carbohydrates - $300

- What distinguishes different blood types?
Carbohydrates - $300

- What distinguishes different blood types?

- Composition of the sugars/glycosylation on the surface of red blood cells
Define each of the following terms:

- Enantiomers
- Anomers
- Diastereomers
- Stereoisomers
- Epimers
Define each of the following terms:

- Enantiomers: stereoisomers that are mirror images of each other
- Anomers: sugars which differ only in the configuration of the anomeric carbon
- Diastereomers: sugars which differ in stereoisomeric configuration
- Stereoisomers: compounds which differ only in the spatial arrangement of their atoms
- Epimers: sugars which differ in configuration of only one carbon
Carbohydrates - $400

- Define each of the following terms:
  - Enantiomers
  - Anomers
  - Diastereomers
  - Stereoisomers
  - Epimers

- Enantiomers: stereoisomers that are mirror images of each other
- Anomers: sugars which differ only in the configuration of the anomeric carbon
- Diastereomers: sugars which differ in stereoisomeric configuration
- Stereoisomers: compounds which differ only in the spatial arrangement of their atoms
- Epimers: sugars which differ in configuration of only one carbon
Where do N-linked and O-linked glycosylation occur on a protein and where do these occur in a cell?
Where do N-linked and O-linked glycosylation occur on a protein and where do these occur in a cell?

- N-linked – attached to the R-group amine of asparagine, occurs in Golgi apparatus and ER
Where do N-linked and O-linked glycosylation occur on a protein and where do these occur in a cell?

- **N-linked** – attached to the R-group amine of asparagine, occurs in Golgi apparatus and ER
- **O-linked** – attached to the R-group hydroxides of serine/threonine, occurs in Golgi apparatus
Serine, aspartic acid, and histididine make up a group of residues in chymotrypsin known as a ____________.
Serine, aspartic acid, and histidine make up a group of residues in chymotrypsin known as a ____________.

Catalytic triad
Proteases - $200

- List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?
List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?

- Serine protease: catalytic triad with a serine (activated to alkoxide ion), covalent intermediate- 2 steps (fast step and slow step)
Proteases - $200

- List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?
  - Serine protease: catalytic triad with a serine (activated to alkoxide ion), covalent intermediate- 2 steps (fast step and slow step)
  - Cysteine protease: cysteine and histidine, covalent intermediate- 2 steps (fast step and slow step)
Proteases - $200

- List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?

  - Serine protease: catalytic triad with a serine (activated to alkoxide ion), covalent intermediate- 2 steps (fast step and slow step)
  - Cysteine protease: cysteine and histidine, covalent intermediate- 2 steps (fast step and slow step)
  - Aspartyl protease: aspartic acids to activate a water molecule and align the peptide for attack, no covalent intermediate holds one of the peptides- 1 step
Proteases - $200

- List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?

  - Serine protease: catalytic triad with a serine (activated to alkoxide ion), covalent intermediate- 2 steps (fast step and slow step)
  - Cysteine protease: cysteine and histidine, covalent intermediate- 2 steps (fast step and slow step)
  - Aspartyl protease: aspartic acids to activate a water molecule and align the peptide for attack, **no** covalent intermediate holds one of the peptides- 1 step
  - Metalloprotease: metal ion to activate a water molecule, **no** covalent intermediate holds one of the peptides- 1 step
Proteases - $200

- List the 4 types of proteases discussed in class. How do these differ in terms of their catalytic mechanisms?

- Serine protease: catalytic triad with a serine (activated to alkoxide ion), covalent intermediate- 2 steps (fast step and slow step)
- Cysteine protease: cysteine and histidine, covalent intermediate- 2 steps (fast step and slow step)
- Aspartyl protease: aspartic acids to activate a water molecule and align the peptide for attack, no covalent intermediate holds one of the peptides- 1 step
- Metalloprotease: metal ion to activate a water molecule, no covalent intermediate holds one of the peptides- 1 step
Proteases - $300

The ____________ is a specialized location in the active site of serine proteases that helps to stabilize the negative charge of the otherwise unstable tetrahedral intermediates.

Bonus: (+$300) name one such enzyme that uses this feature.
The ___________ is a specialized location in the active site of serine proteases that helps to stabilize the negative charge of the otherwise unstable tetrahedral intermediates.

Bonus: (+$300) name one such enzyme that uses this feature.

- Oxyanion hole
The __________ is a specialized location in the active site of serine proteases that helps to stabilize the negative charge of the otherwise unstable tetrahedral intermediates.

- Bonus: (+$300) name one such enzyme that uses this feature.

- Oxyanion hole
- Bonus: chymotrypsin
Chymotrypsin uses an active site serine to carry out its chemistry. Name two other categories or major classes of proteases.

Bonus (+$200): Name a third category or major class of protease.
Chymotrypsin uses an active site serine to carry out its chemistry. Name two other categories or major classes of proteases.

- **Bonus (+$200):** Name a third category or major class of protease.
  - Cysteine Proteases ex. Papain
  - Aspartyl Proteases ex. Renin
  - Metalloproteases ex. Thermolysin
Proteases - $500

- Enzymes other than chymotrypsin, like trypsin and elastase, use an active site serine and catalytic triad. Why do their substrate specificities differ?

- Bonus: Describe this feature for one serine protease and how this corresponds to its specificity.
Enzymes other than chymotrypsin, like trypsin and elastase, use an active site serine and catalytic triad. Why do their substrate specificities differ?

Bonus: Describe this feature for one serine protease and how this corresponds to its specificity.

Differ by the “specificity pocket (S1)” for binding their substrates.
Enzymes other than chymotrypsin, like trypsin and elastase, use an active site serine and catalytic triad. Why do their substrate specificities differ?

Bonus: Describe this feature for one serine protease and how this corresponds to its specificity.

Differ by the “specificity pocket (S1)” for binding their substrates.

Bonus: ex. Trypsin has a negatively charged amino acid at the bottom of its S1 pocket so it can favorably interact with positively charged amino acids arginine and lysine (and as a result cuts after arginine and lysine)
How is the β-adrenergic receptor signaling pathway turned off?
How is the $\beta$-adrenergic receptor signaling pathway turned off?

- Receptor: if the receptor gets stuck, it is phosphorylated by the G-protein receptor kinase which provides a binding site for arrestin. Arrestin prevents G-protein from interacting with the receptor, stopping signaling by the G-protein.
How is the β-adrenergic receptor signaling pathway turned off?

- Receptor: if the receptor gets stuck, it is phosphorylated by the G-protein receptor kinase which provides a binding site for arrestin. Arrestin prevents G-protein from interacting with the receptor, stopping signaling by the G-protein.

- G-protein: auto-regulating, alpha subunit hydrolyzes GTP to GDP and re-binds to the beta and gamma units and no longer associates with adenylate cyclase (adenylate cyclase no longer activated)
How is the β-adrenergic receptor signaling pathway turned off?

- **Receptor**: if the receptor gets stuck, it is phosphorylated by the G-protein receptor kinase which provides a binding site for arrestin. Arrestin prevents G-protein from interacting with the receptor, stopping signaling by the G-protein.

- **G-protein**: auto-regulating, alpha subunit hydrolyzes GTP to GDP and re-binds to the beta and gamma units and no longer associates with adenylate cyclase (adenylate cyclase no longer activated)

- **cAMP**: phosphodiesterase breaks down cAMP
How is the β-adrenergic receptor signaling pathway turned off?

- Receptor: if the receptor gets stuck, it is phosphorylated by the G-protein receptor kinase which provides a binding site for arrestin. Arrestin prevents G-protein from interacting with the receptor, stopping signaling by the G-protein.
- G-protein: auto-regulating, alpha subunit hydrolyzes GTP to GDP and re-binds to the beta and gamma units and no longer associates with adenylate cyclase (adenylate cyclase no longer activated)
- cAMP: phosphodiesterase breaks down cAMP
- PKA: breakdown of cAMP by phosphodiesterase has the effect of inactivating protein kinase A.
Signaling - $200

- What is the result of insulin signaling?
- Bonus: What kind of receptor does insulin bind to?
What is the result of insulin signaling?

Bonus: What kind of receptor does insulin bind to?

Insulin signaling involves a kinase/phosphorylation cascade. Insulin results in the movement of the GLUT4 protein to the cell surface. GLUT4 is a glucose transport protein that transports glucose into cells.
What is the result of insulin signaling?

Bonus: What kind of receptor does insulin bind to?

Insulin signaling involves a kinase/phosphorylation cascade. Insulin results in the movement of the GLUT4 protein to the cell surface. GLUT4 is a glucose transport protein that transports glucose into cells.

Bonus: a receptor tyrosine kinase
What is the result of insulin signaling?

Bonus: What kind of receptor does insulin bind to?

Insulin signaling involves a kinase/phosphorylation cascade. Insulin results in the movement of the GLUT4 protein to the cell surface. GLUT4 is a glucose transport protein that transports glucose into cells.

Bonus: a receptor tyrosine kinase
Signaling - $300

- Describe the mechanism of activation of G proteins.
Signaling- $300

- Describe the mechanism of activation of G proteins.
  
  - A change in shape of the receptor causes changes in the G protein.
Describe the mechanism of activation of G proteins.

- A change in shape of the receptor causes changes in the G protein.
- In the G protein, the $\alpha$ subunit binds GDP but when activated, GDP is exchanged for GTP; $\alpha$ subunit simultaneously dissociates from $\beta$ and $\gamma$. 
Signaling- $300

- Describe the mechanism of activation of G proteins.
  - A change in shape of the receptor causes changes in the G protein.
  - In the G protein, the α subunit binds GDP but when activated, GDP is exchanged for GTP; α subunit simultaneously dissociates from β and γ
  - Activated G protein transmits signals by binding other proteins (here, adenylate cyclase)
Signaling- $400

- Describe the steps of signaling through the beta-adrenergic receptor pathway.
- Bonus: What is the second messenger? What is the first messenger?
Describe the steps of signaling through the beta-adrenergic receptor pathway.

Bonus: What is the second messenger? What is the first messenger?

1) binding of hormone by receptor; 2) activation of G-protein by replacement of GDP by GTP in the alpha subunit; 3) binding of the alpha subunit (with GTP) by adenylate kinase; 4) production of cAMP by adenylate cyclase; 5) activation of protein kinase A by binding of cAMP; 6) phosphorylation of glycogen synthase (inactivates) and phosphorylase kinase (activates) by protein kinase A; 7) phosphorylation of glycogen phosphorylase by phosphorylase kinase (activates it); and 8) production of glucose-1-phosphate by action of glycogen phosphorylase on glycogen.
Describe the steps of signaling through the beta-adrenergic receptor pathway.

Bonus: What is the second messenger? What is the first messenger?

1) binding of hormone by receptor; 2) activation of G-protein by replacement of GDP by GTP in the alpha subunit; 3) binding of the alpha subunit (with GTP) by adenylate kinase; 4) production of cAMP by adenylate cyclase; 5) activation of protein kinase A by binding of cAMP; 6) phosphorylation of glycogen synthase (inactivates) and phosphorylase kinase (activates) by protein kinase A; 7) phosphorylation of glycogen phosphorylase by phosphorylase kinase (activates it); and 8) production of glucose-1-phosphate by action of glycogen phosphorylase on glycogen.

Bonus: second messenger: cAMP; first messenger: hormone
Signaling- $500

- Describe RAS and its role in signaling.
- Bonus: What are mutations in RAS often associated with?
Signaling - $500

- Describe RAS and its role in signaling.
- Bonus: What are mutations in RAS often associated with?

- RAS is a protein that plays a role in the signaling process, a proto-oncogene.
- RAS is involved in EGF signaling. The signaling complex that forms after EGF binding and activation of the EGF receptor activates RAS by causing it to release GDP and replace it with GTP. (When bound to GDP, it is inactive and when it binds to GTP, it is activated.)
- Activation of RAS results in activation of transcription pathways that result in cell division.
- Mutations to RAS result in RAS being left on (so cells continuously divide). Mutated RAS is the most common point mutation in cancer - found in 90% of pancreatic cancers and 20% of all cancers.
RAS is a protein that plays a role in the signaling process, a proto-oncogene. RAS is involved in EGF signaling. The signaling complex that forms after EGF binding and activation of the EGF receptor activates RAS by causing it to release GDP and replace it with GTP. (When bound to GDP, it is inactive and when it binds to GTP, it is activated.) Activation of RAS results in activation of transcription pathways that result in cell division. Mutations to RAS result in RAS being left on (so cells continuously divide). Mutated RAS is the most common point mutation in cancer - found in 90% of pancreatic cancers and 20% of all cancers.
Describe RAS and its role in signaling.

Bonus: What are mutations in RAS often associated with?

- RAS is a protein that plays a role in the signaling process, a proto-oncogene.
- RAS is involved in EGF signaling. The signaling complex that forms after EGF binding and activation of the EGF receptor activates RAS by causing it to release GDP and replace it with GTP. (When bound to GDP, it is inactive and when it binds to GTP, it is activated.)
- Activation of RAS results in activation of transcription pathways that result in cell division.
- Mutations to RAS result in RAS being left on (so cells continuously divide). Mutated RAS is the most common point mutation in cancer - found in 90% of pancreatic cancers and 20% of all cancers.
Signaling- $500

- Describe RAS and its role in signaling.
- Bonus: What are mutations in RAS often associated with?

- RAS is a protein that plays a role in the signaling process, a proto-oncogene.
- RAS is involved in EGF signaling. The signaling complex that forms after EGF binding and activation of the EGF receptor activates RAS by causing it to release GDP and replace it with GTP. (When bound to GDP, it is inactive and when it binds to GTP, it is activated.)
- Activation of RAS results in activation of transcription pathways that result in cell division.
- Mutations to RAS result in RAS being left on (so cells continuously divide). Mutated RAS is the most common point mutation in cancer - found in 90% of pancreatic cancers and 20% of all cancers.
The activity of chymotrypsin can be studied using an artificial substrate which, when cleaved by the enzyme, releases a yellow product. When the release of the colored substrate by the enzyme is studied, there is a VERY rapid release of the colored substrate. After that initial burst of activity, the remaining yellow color is released slowly. Explain this observation.
The activity of chymotrypsin can be studied using an artificial substrate which, when cleaved by the enzyme, releases a yellow product. When the release of the colored substrate by the enzyme is studied, there is a VERY rapid release of the colored substrate. After that initial burst of activity, the remaining yellow color is released slowly. Explain this observation.
The activity of chymotrypsin can be studied using an artificial substrate which, when cleaved by the enzyme, releases a yellow product. When the release of the colored substrate by the enzyme is studied, there is a VERY rapid release of the colored substrate. After that initial burst of activity, the remaining yellow color is released slowly. Explain this observation.

The reaction catalyzed occurs in two steps. The first step cleaves the bond to produce the yellow product, which is rapidly released. The other product of this reaction is the remainder of the substrate that is covalently linked to the enzyme. In order for the enzyme to bind another substrate molecule and release more yellow color, it must first release the covalently bound molecule. This step occurs slowly and explains why subsequent yellow molecules are released slowly - after the initial one is released, the enzyme must remove the covalently bound molecule, bind a new substrate, and cut the substrate and the continue the process repeatedly.