

Bio 111 Handout for Biochemistry 1

This handout contains:

1. Today's iClicker Questions
2. The handout for today's lecture.

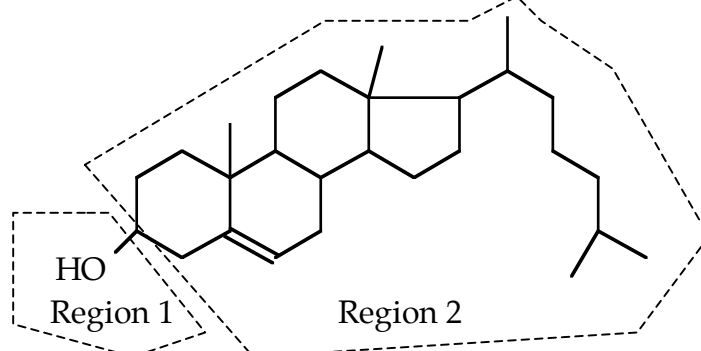
iClicker Question #13A - before lecture

Your lunch consists primarily of polymers. Digestion breaks these down into monomers. These monomers are then re-polymerized to make more of you. Which of the following statements is/are true?

- A) Digestion is dehydration synthesis; re-polymerization is hydrolysis.
- B) Digestion is dehydration synthesis; re-polymerization is dehydration synthesis.
- C) Digestion is hydrolysis; re-polymerization is dehydration synthesis.
- D) Digestion is hydrolysis; re-polymerization is hydrolysis.
- E) None of the above.

iClicker Question #13B - after lecture

The structure of cholesterol is shown below with two regions circled:



Cholesterol is found embedded in the membrane of animal cells. Based on this, which of the following statements is/are true?

- A) Region (1) would be in the hydrophobic core of the membrane and (2) would be in the surrounding water.
- B) Region (2) would be in the hydrophobic core of the membrane and (1) would be in the surrounding water.
- C) Both (1) and (2) would be in the water surrounding the membrane.
- D) Both (1) and (2) would be in the hydrophobic core of the membrane.
- E) I don't know.

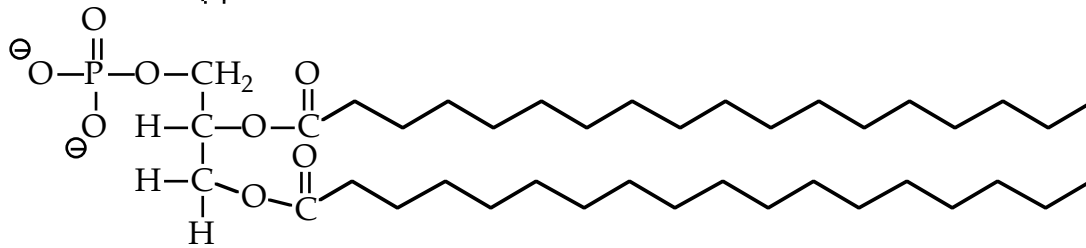
Beaming in your answers

1. Figure out your answer and select the appropriate letter (A-E).
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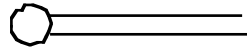
lipids a phospholipid

hydrophilic "head"

hydrophobic "tail"



schematically:



Bio 111 Handout for Biochemistry 2

This handout contains:

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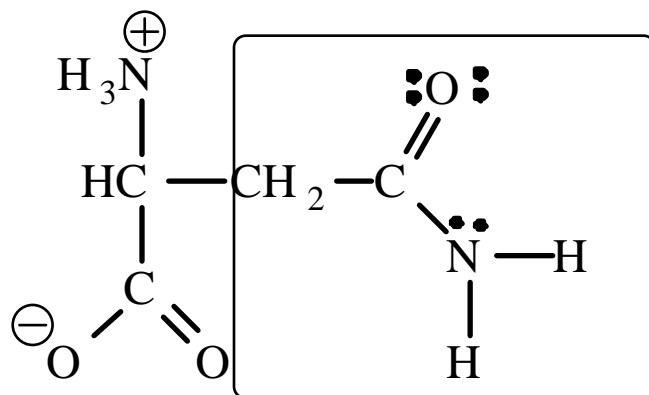
iClicker Question #14A - before lecture

Consider the table of amino acid structures in Campbell figure 5.17. Which of the following amino acids has the most hydrophobic side chain?

- A) Alanine
- B) Phenylalanine
- C) Aspartic acid
- D) Glycine
- E) Serine

iClicker Question #14B - after lecture

Consider the amino acid asparagine. Its structure is shown below with the side chain circled.



Which of the following statements about the side chain of asparagine are correct?

- (A) The side chain is hydrophilic & it can make H-bonds.
- (B) The side chain is hydrophobic & it can make ionic bonds.
- (C) The side chain is hydrophilic & it can make ionic and H-bonds.
- (D) The side chain is hydrophilic and it can make ionic bonds.
- (E) I don't know.

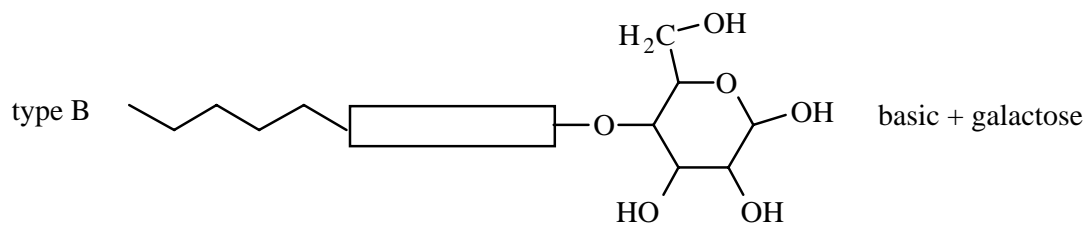
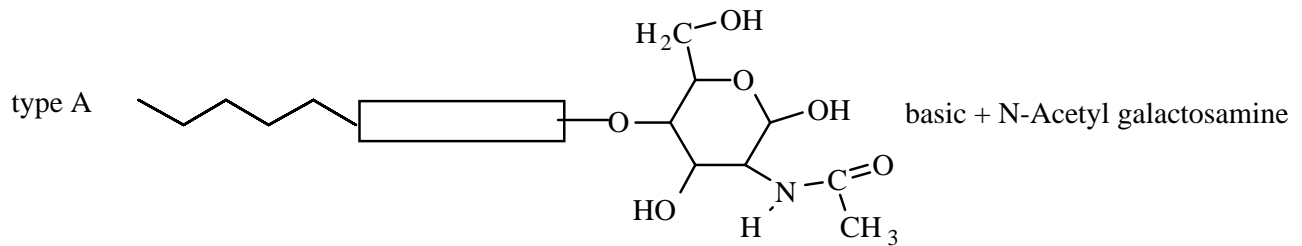
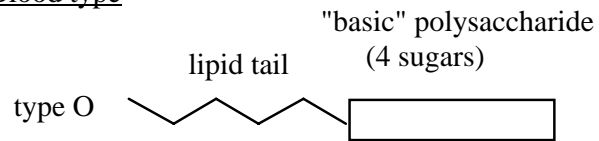
Beaming in your answers

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Bio 111 Macromolecules Handout

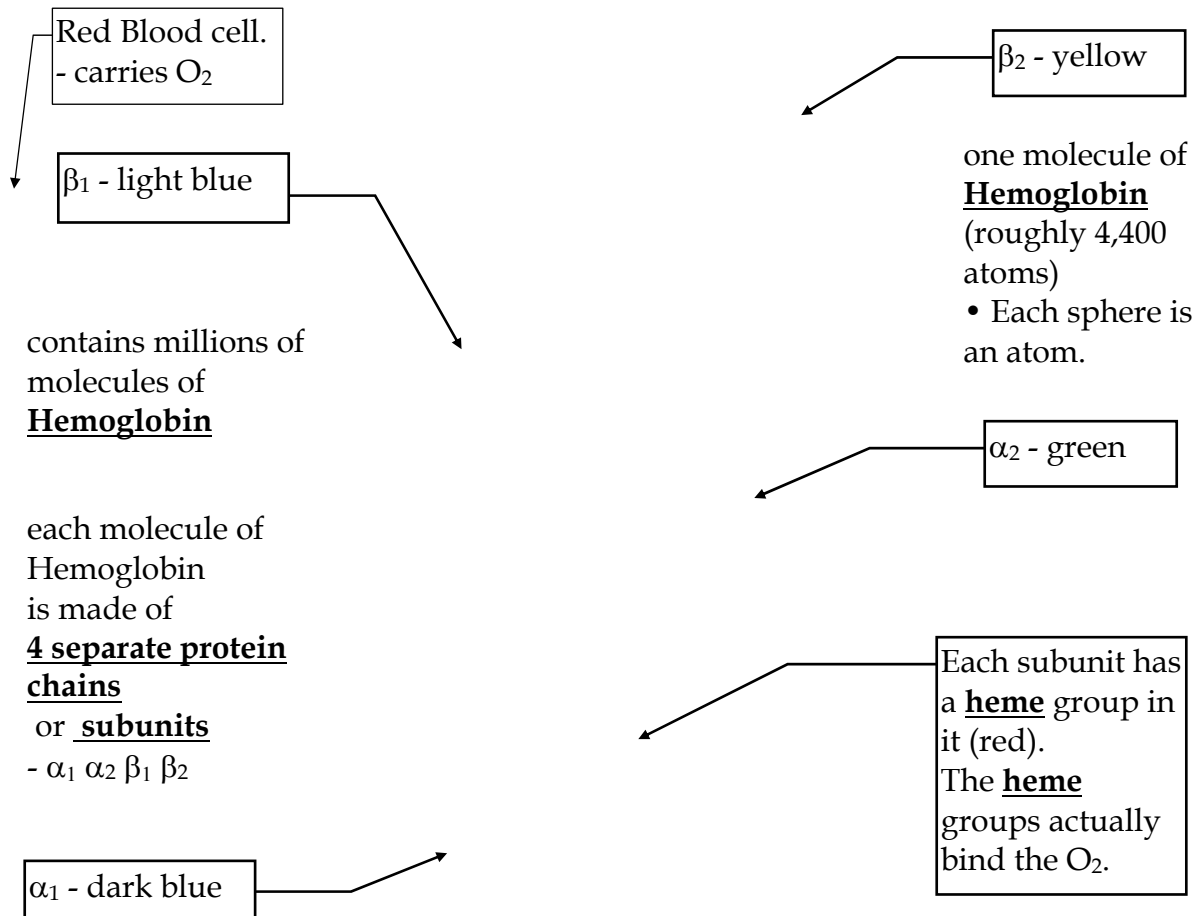
Blood type



type AB = mixture of BOTH A and B

Hemoglobin Protein Structure

All of these figures can be found on the web by downloading the "Lecture Molecules Application" from the course web site.



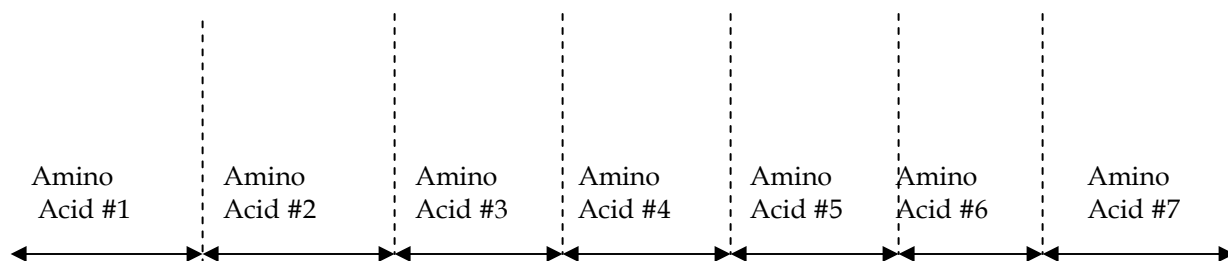
The **heme** groups are embedded in the protein; each binds one O₂. Since there are 4 subunits in a hemoglobin molecule, each hemoglobin molecule binds 4 O₂.

Bio 111: Protein Folding Demonstration

Today in lecture, you will twist a piece of wire so that it simulates the behavior of a chain of amino acids (also known as a protein). We will then use this to explore protein folding in lecture.

A simulated 7-amino acid protein is shown below; your protein will be similar although it may have a different pattern of loops.

- The more-or-less straight part of the wire simulates the **backbone**
- The loops simulate the **side-chains** (there are 7 in this example)
- One end is the amino terminus; the other is the carboxyl terminus (for this demonstration, it does not matter which is which).
-



Side-chains

In the simplified world of this demonstration, there are three kinds of amino acids, each of which is represented by a different type of loop:

| <u>Hydrophobic</u> | <u>Positively-charged</u> | <u>Negatively-charged</u> |
|--|--|--|
| | | |
| A <u>large open loop</u> . Make it two finger-widths wide. | A <u>long closed loop</u> . Make it <u>four</u> twists long. | A <u>short closed loop</u> . Make it <u>two</u> twists long. |

How to do it

Now you will twist your wire to make a protein of seven or eight amino acids. The sequence of amino acids is up to you, but it works best if your protein contains:

- More hydrophobic amino acids than charged ones.
- At least one positively-charged and one negatively-charged amino acid.

The measurements in this demonstration need not be precise; you will use the width of your fingers as a ruler.

- Leave two finger-widths of space un-twisted at the starting end.
- Leave three finger-widths of space between each amino acid.

Bio 111 Handout for Biochemistry 3

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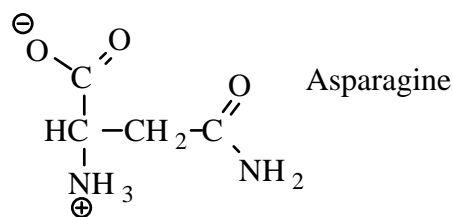
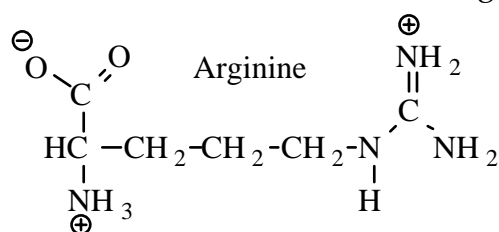
iClicker Question #15A - before lecture

Campbell figure 5.20 shows the four levels of protein structure. All proteins can be looked at in terms of three of the levels of structure; only some proteins can be looked at in terms of one of the levels. Which level applies to only some proteins?

- (A) Primary Structure
- (B) Secondary Structure
- (C) Tertiary Structure
- (D) Quaternary Structure
- (E) None of the above

iClicker Question #15B - after lecture

Consider the two amino acids, Arginine and Asparagine (shown below).



Which interactions are possible between the side chains of these two amino acids?

- (A) ionic, hydrogen, van der Waals, and hydrophobic
- (B) hydrogen, van der Waals, and hydrophobic
- (C) van der Waals and hydrophobic
- (D) ionic and van der Waals
- (E) hydrogen and van der Waals

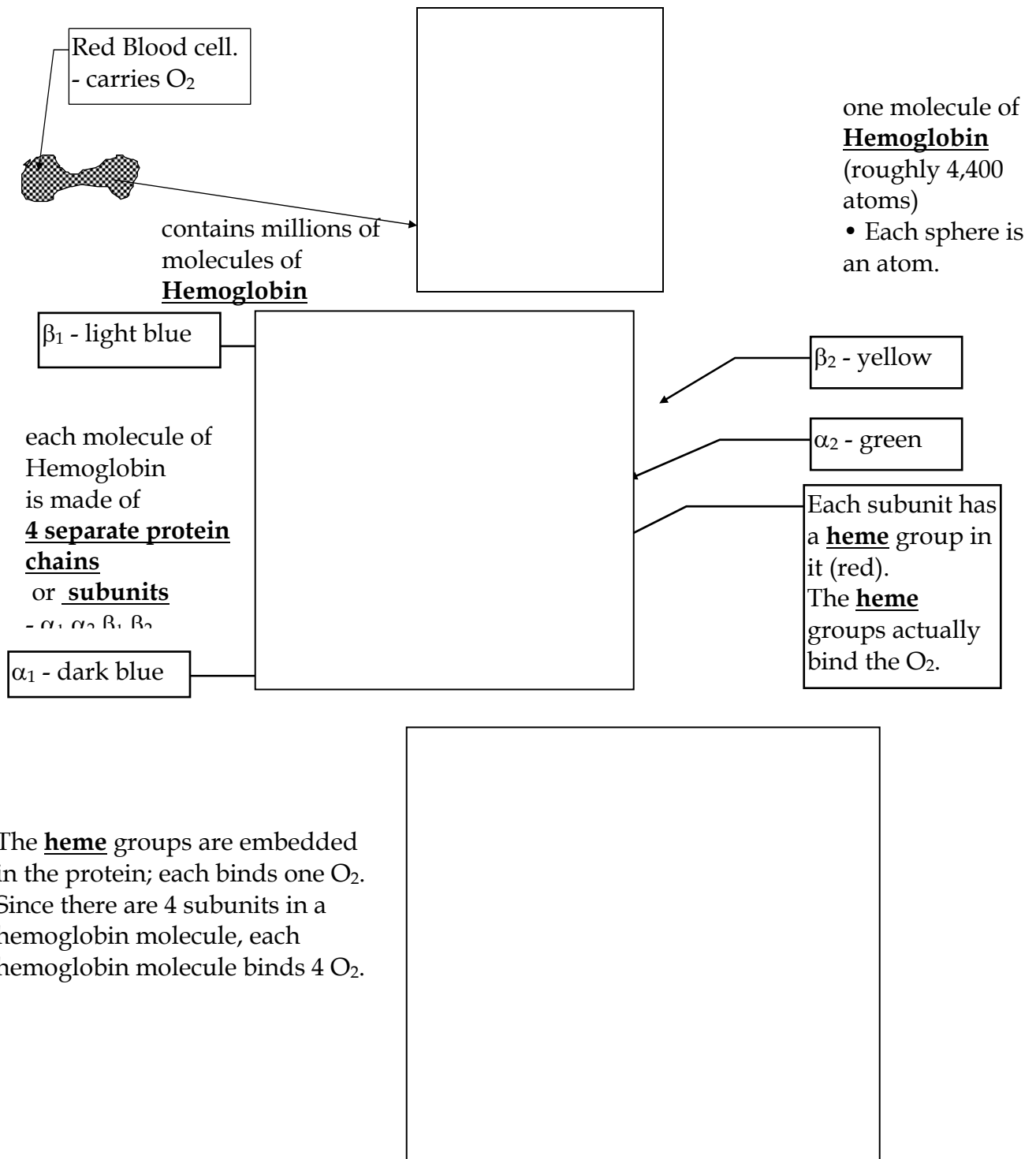
Beaming in your answers

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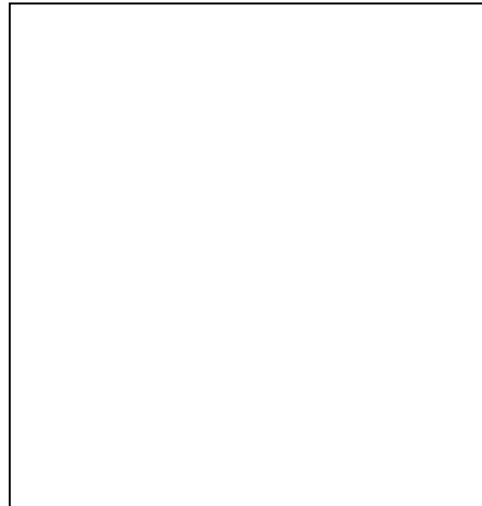
Hemoglobin Protein Structure

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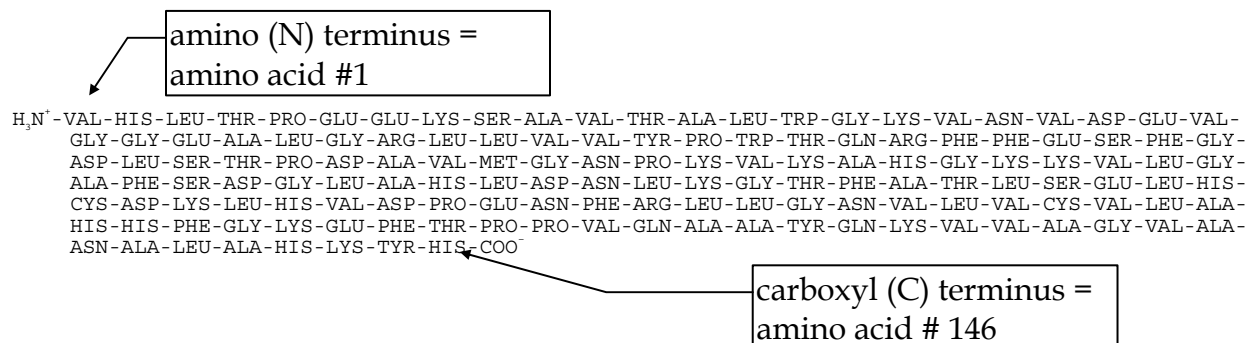


Focus in on one subunit: β-globin ("beta globin")

- 146 amino acids linked in a single unbranched chain.
- also includes one heme
- roughly 1200 atoms



(1) Primary Structure (1° structure) = sequence of amino acids ("amino acid sequence")



(2) Secondary Structure (2° structure) = major backbone features (α-helix & β-sheet)

One way to simplify a complex protein is to look at it as three parts:

- the **backbone** (shown as thick pink & white lines). This is the same for all amino acids.
- the **side-chains** (shown as thin blue lines). These are different for each kind of amino acid.
- the **heme** (shown in red). This binds O₂.



amino (N) terminus = amino acid #1

This shows the **backbone and heme**.

- the pink parts of the backbone are α -helix.
- β -globin has no regions that are in β -sheet configuration.

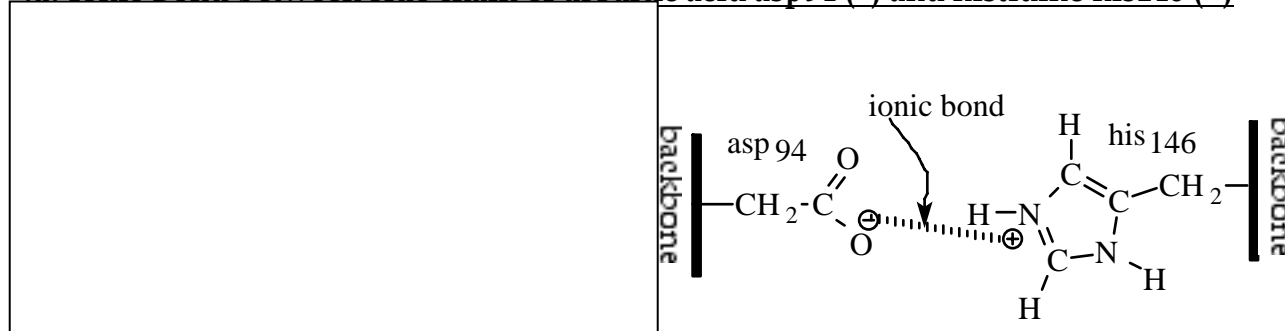
carboxyl (C) terminus = amino acid #146

A close-up of a region of **α -helix**. Showing only the backbone.

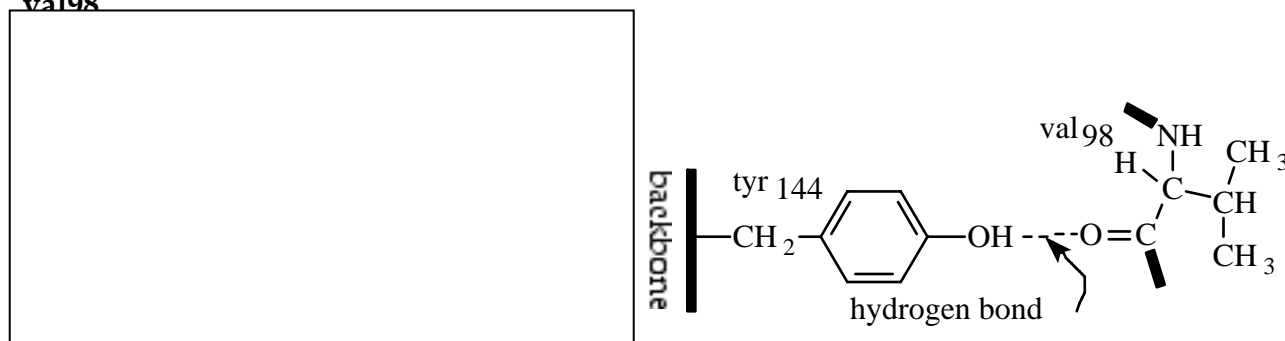
The same region of **α -helix**. Showing the backbone atoms and hydrogen-bonds (shown as dotted lines) that hold the helix in shape.

(3) Tertiary Structure (3° structure) = **interactions between side-chains**
(ionic bonds, hydrogen bonds, and hydrophobic interactions)

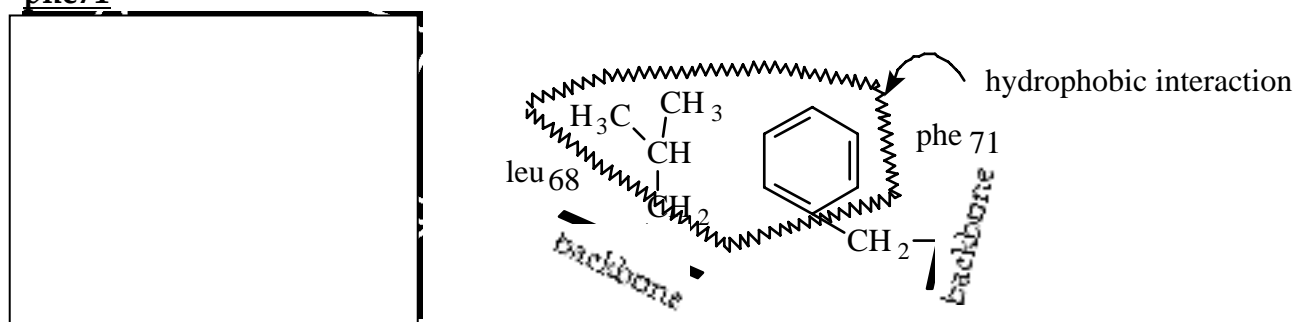
(a) Ionic Bond between side chain of aspartic acid asp94 (-) and histidine his146 (+)



(b) hydrogen bond between the OH on the side chain of tyrosine tyr145 and O=C of Valine val98



(c) hydrophobic interaction between the side chains of leucine leu68 and phenylalanine phe71



(d) the interior ("core") of the protein is mostly hydrophobic amino acids

This is a cross section of the molecule - a slice through the middle.

- Hydro~~phobic~~**phobic** amino acids are shown in **red**.
- Hydro~~philic~~**philic** amino acids are shown in **white**.

The hydrophobic effect 'forces' the 'phobics' into the center, helping to hold the protein in its

(4) Quaternary Structure (4^o structure) = **interactions between more than one protein chain.**

Each molecule of hemoglobin is made of 4 subunits, 2 α 's (yellow and light blue) and 2 β 's (blue and green) - this is an example of 4^o structure.

Each subunit also has a heme group (red).

Here is one subunit, a β -globin.

The blue parts are where the β contacts one α subunit. **The subunits are held to each other via hydrogen bonds, ionic bonds, etc.**

The green parts are where the β contacts the other α subunit.

Bio 111 Handout for Biochemistry 4

This handout contains:

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iClicker Question #16A - before lecture

Figure 5.21 in Campbell describes the mutation that results in Sickle Cell Anemia.

Which of the following statements is true?

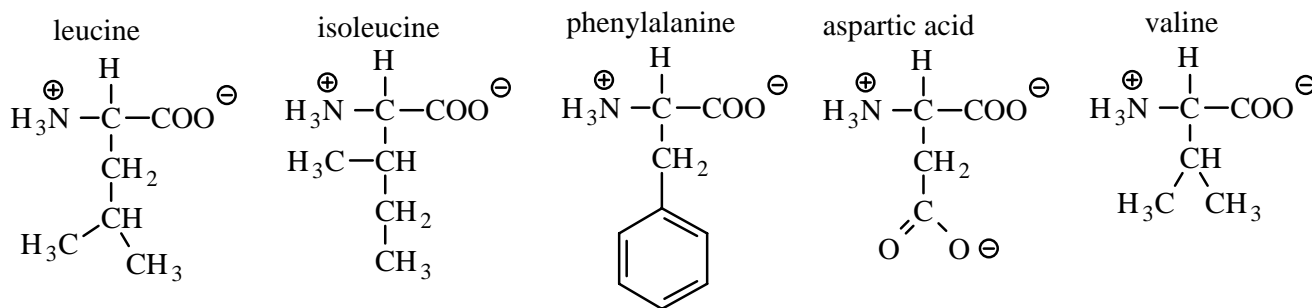
- (A) When sickle cell hemoglobin is polymerized, a valine is added to the chain at position 6 rather than a glutamic acid.
- (B) When sickle cell hemoglobin is first polymerized, amino acid 6 is glutamic acid, as it is in normal hemoglobin. The mutation then changes the amino acid at position 6 in the protein into valine.
- (C) I don't know.

iClicker Question #16B - after lecture

Suppose the normal amino acid at position 46 in a protein was leucine; the side chain of 46 is deep in the core of the protein. Which of the following substitutions would be likely to have the greatest effect on the protein's structure?

- (A) changing leucine 46 to isoleucine
- (B) changing leucine 46 to phenylalanine
- (C) changing leucine 46 to aspartic acid
- (D) changing leucine 46 to valine
- (E) I don't know.

The structures of the relevant amino acids are shown below



Beaming in your answers

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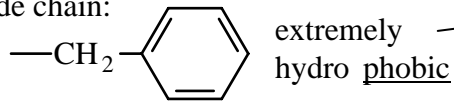
- b. The “STATUS” light will flash green to indicate that your answer has been received. If the “STATUS” light flashed red, your answer was not received; you should re-send it until you get a green “STATUS” light.

Mutant Forms of Hemoglobin

(1) Hemoglobin Christchurch (Hb^{Christchurch})

- In normal hemoglobin, amino acid 71 of the β -subunits is buried deep in the hydrophobic core of the protein and is phenylalanine:

side chain:

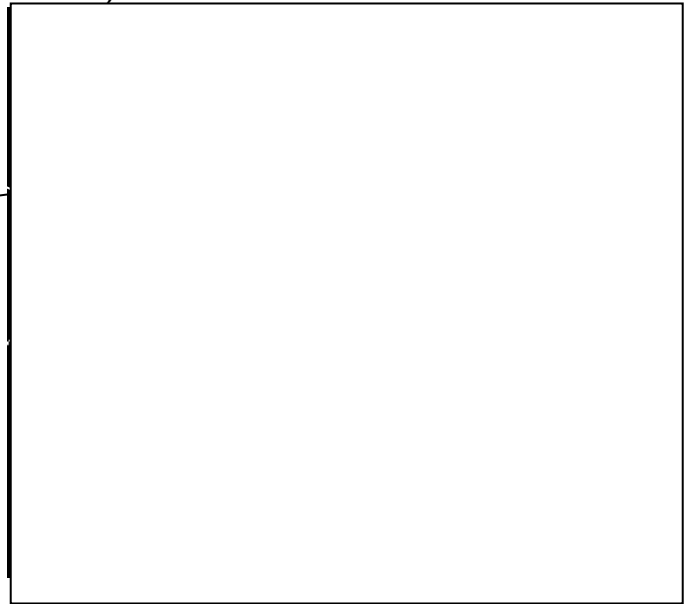


- In Hb^{Christchurch}, amino acid #71 is serine:

side chain:



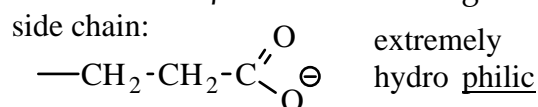
Hb^{Christchurch} is unstable and forms Heintz Bodies in red blood cells.



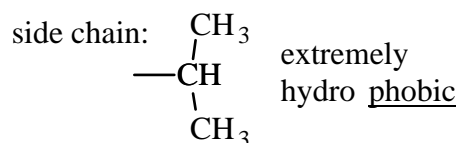
(2) Sickle-cell hemoglobin (Hb_S)

- Normal hemoglobin molecules do not stick to each other.
- Hb_S molecules stick together under conditions of low O₂:

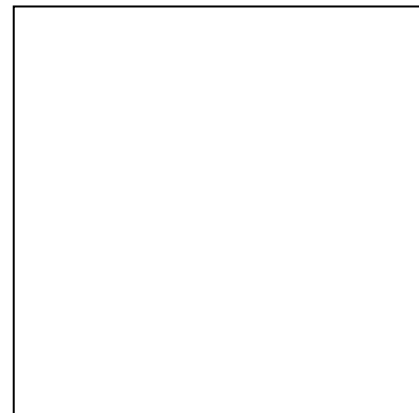
- In normal hemoglobin, amino acid #6 is on the outside of the β -subunits and is glutamic acid:

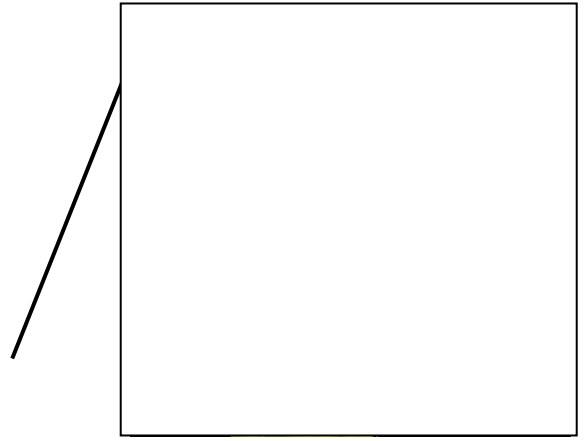


- In Hb_S, amino acid #6 of the β -subunits is valine:

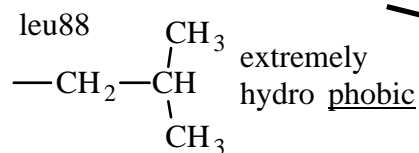
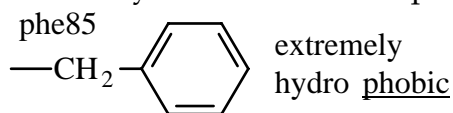


This produces a hydrophobic spot on the surface of the β -subunits of Hb_S.

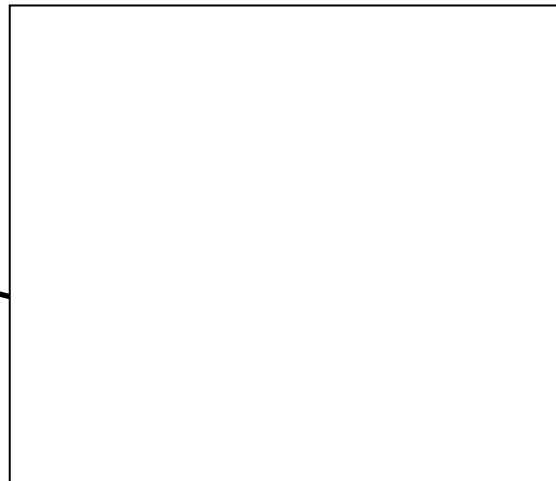




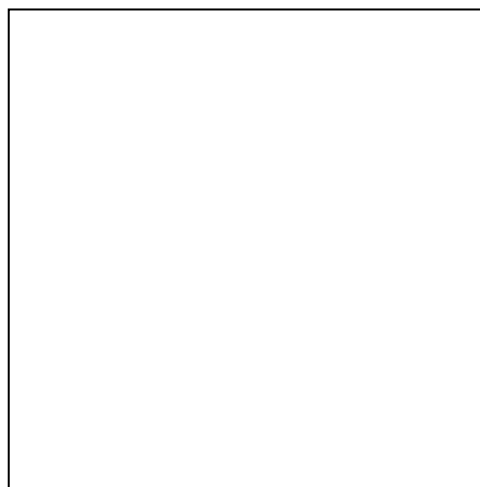
In both Hb_s and normal Hb, there is a small hydrophobic patch on the surface of all β -subunits formed by the side chains of phe85 and leu88:



This patch is exposed when O₂ levels are low.



- When O₂ levels are low, these 2 hydrophobic patches stick together via the hydrophobic effect.
- This causes Hb_s molecules to stick together in long chains.
- These long chains distort the red blood cells and cause them to form “sickle” shapes.

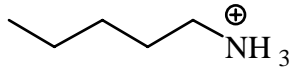


(3) Hemoglobin Woolwich

(Hb_{Woolwich})

- In normal hemoglobin, amino acid 132 of the β -subunit is on the surface of the protein and is lysine.

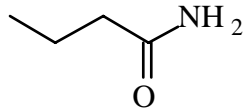
side chain:



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

- In Hb Woolwich, amino acid 132 is glutamine:

side chain:



Hb Woolwich is non-functional but the protein molecules do not form Heintz bodies. They are freely-soluble just like normal hemoglobin, but they do not bind oxygen.

Bio 111 Handout for Biochemistry 5

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iClicker Question #17A - before class

When you burn a piece of paper, energy is released in the form of heat and light. Where did this energy come from?

- (A) chemical energy in the paper
- (B) solar energy used by the plant to make the paper
- (C) energy from nuclear fusion in the sun
- (D) all of the above
- (E) none of the above

iClicker Question #17B - after class

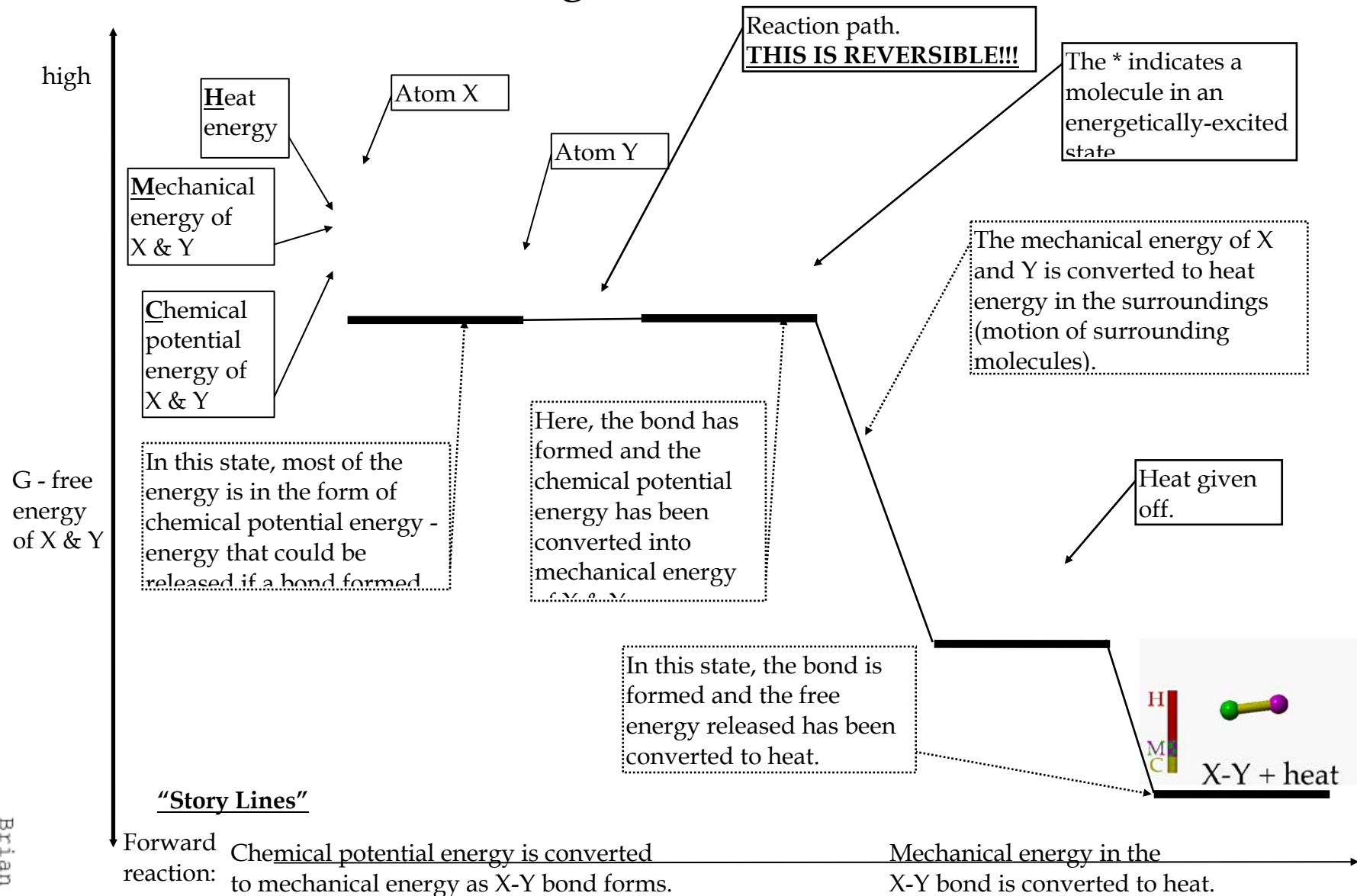
In liquid H₂O, each H₂O molecule is tightly attached to neighboring H₂O molecules by a network of hydrogen bonds. In steam (gaseous water), the H₂O molecules act as individuals and are not H-bonded to anything. Thus, boiling water involves breaking these H-bonds.

- a) the ΔG for the reaction (liquid water) \Rightarrow (steam) is -; that is, the $\Delta G < 0$
 - b) the ΔG for the reaction (liquid water) \Rightarrow (steam) is +; that is, the $\Delta G > 0$
 - c) the ΔG for the reaction (steam) \Rightarrow (liquid water) is -; that is, the $\Delta G < 0$
- (A) Statement (a) is FALSE; all the others are TRUE.
 - (B) Statement (b) is FALSE; all the others are TRUE.
 - (C) Statement (c) is FALSE; all the others are TRUE.
 - (D) More than one statement is FALSE
 - (E) I don't know.

Beaming in your answers

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Bond Formation/Breaking: $X + Y \rightleftharpoons X-Y$



Mechanical energy is converted to chemical
potential energy as X-Y is broken.

Heat is converted to mechanical
energy in the X-Y bond.

Bio 111 Handout for Biochemistry 6

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3. Information for Exam 2.

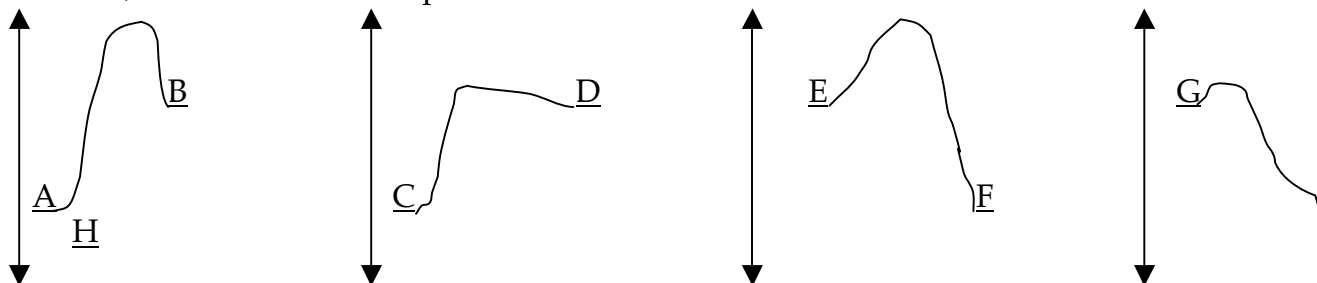
iClicker Question #18A - before lecture

Which of the following statements about catalysts are true?

- (A) Catalysts increase the rate of a reaction.
- (B) Catalysts are not permanently altered by the reaction they catalyze.
- (C) Catalysts do not change the ΔG of a reaction.
- (D) All of the above.
- (E) None of the above.

iClicker Question #18B - after lecture

Consider the following free energy diagrams. Assuming that all 4 are drawn to the same scale, which reaction will proceed the FASTEST?



- (A) $A \Rightarrow B$ will be fastest
- (B) $C \Rightarrow D$ will be fastest
- (C) $E \Rightarrow F$ will be fastest
- (D) $G \Rightarrow H$ will be fastest
- (E) I don't know.

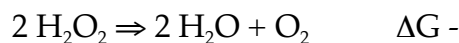
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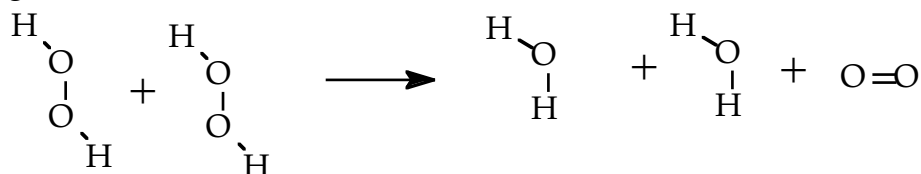
Bio 111 Thermodynamics II

Today, we will be dealing with a chemical reaction that occurs both in the absence of living things and inside living cells. We will begin by looking at how the reaction proceeds without catalysis.

The reaction is the breakdown of two molecules of hydrogen peroxide (H_2O_2) into two molecules of water (H_2O) and one molecule of oxygen gas (O_2). This is a spontaneous reaction; you know this because hydrogen peroxide you buy at the drugstore 'goes bad' (breaks down) over time. Therefore:



Looking at the bonds made and broken:



Bonds at start

4 O-H

2 O-O

Bonds at end

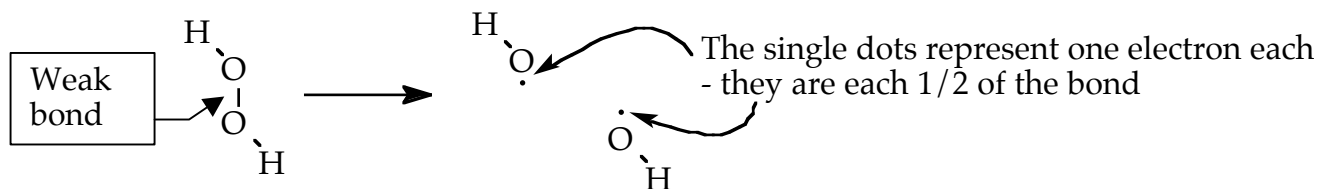
4 O-H

1 O=O

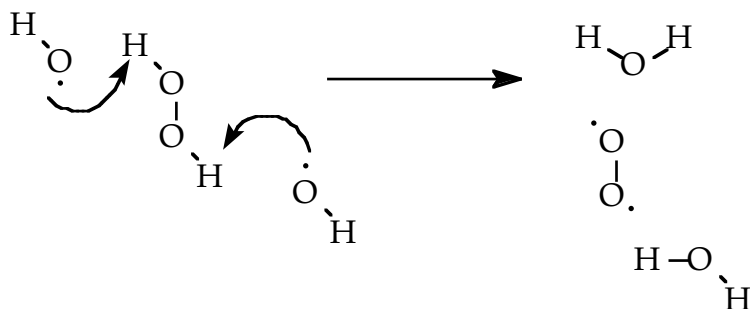
So the net change is to break 2 O-O single bonds and replace them with one O=O double bond. Since O-O bonds are particularly weak (the lone pairs repel each other) and an O=O double bond is particularly strong, the ΔG for this reaction is negative.

Without a catalyst, this reaction proceeds in the following steps (simplified):

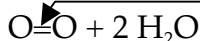
(1) Break the O-O bond (takes energy input ΔG^+) **this is the TRANSITION STATE:**



(2) The $\text{H-O}\cdot$ fragments then attack another H_2O_2 , taking away 2 $\text{H}\cdot$'s ($\Delta G \sim 0$):

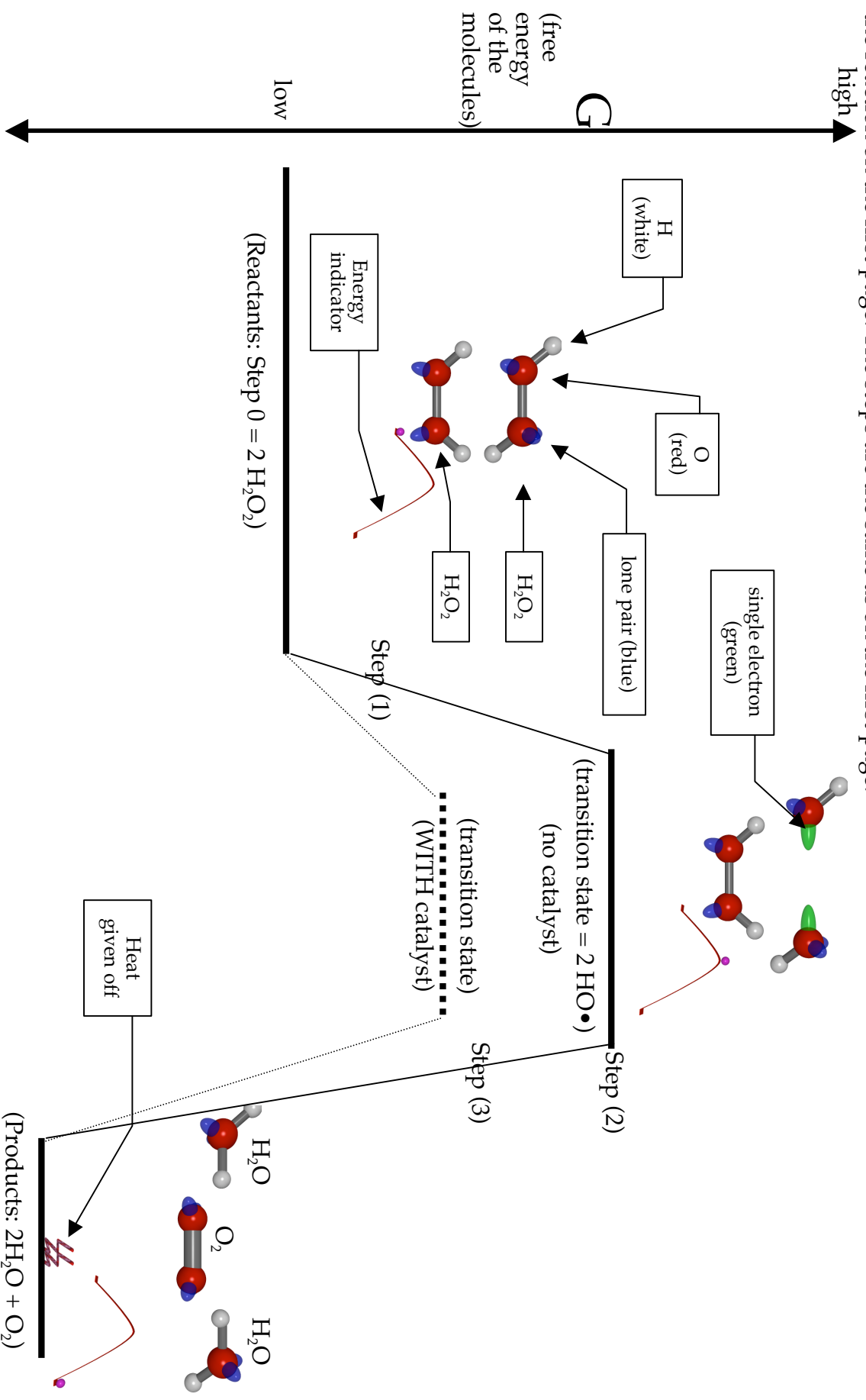


(3) The $\frac{1}{2}$ bonds (\cdot) on the O-O join to form a second O-O bond (ΔG^-) to give the final products:



Strong bond

Overall, a weak bond has been exchanged for a strong bond so the reaction is spontaneous. **However**, forming the transition state is hard (=high activation energy), so the reaction is slow. This is a free-energy diagram of the reaction on the first page. The steps are the same as on the first page.



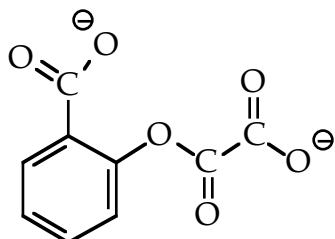
Bio 111: Information for Exam II

Basic Facts

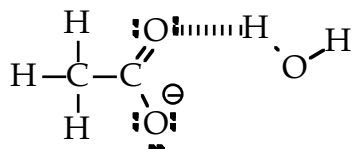
- The exam will be held in Lipke {see syllabus for dates} from 12:30 to 1:20.
- The exam will cover from Chemistry 1 through Biochemistry 8
- The exam will consist of approximately 4 or 5 questions. These will not be multiple choice; they will be problem-solving. A typical problem starts with a simple question, and then gets harder.
- A copy of an old version of Exam II can be found near the back of the Lab Manual; solutions can be found in this handout. **There will be no cell biology question on this year's exam.**
 - You will need to be able to work problems like those that were assigned in Chapter 2 of APAIB, **except**: 3.3.1, 3.3.3, 3.3.4, and 3.3.7.
 - You need to know:
 - how to draw a chemical structure that follows all the bonding rules
 - how to look at a chemical structure and
 - tell if the structure is correct (right # of bonds & charges)
 - tell which parts are hydrophobic/hydrophilic (rank in order)
 - what type of bonds each part can make (ionic, H-bond (& how to draw one), 'phobic, & relative strengths)
 - levels of protein structure & forces that govern their formation
 - how to interpret & explain effects of amino acid changes
 - how to design and interpret a binding site like you did in the Protein Structure Lab Report
 - how to apply the “doing something is dominant to not doing something” rule from Biochem 4 and Biochem 7
 - how a blockage in a pathway can lead to substrate buildup (like in Biochem 7 and Question 5 from the Glycolysis lab)
 - ΔG , coupled reactions, rate, activation energy, catalysis
 - the general features of fig. 9.16 (not the numbers), and fig. 10.21
 - be able to briefly describe a chemical transformation given the reactants and products (like Question 2 in the Glycolysis Lab)
 - $\text{ATP} \Rightarrow \text{ADP} + \text{P}_i$; which is high/low energy; what you can do with ATP, how it's made; the ATP cycle from lecture)
 - $\text{NAD}^+ \Rightarrow \text{NADH} + \text{H}^+$; what it carries; how it can be re-cycled, how many ATP you can get from each NADH in electron transport.
 - You **do not** need to know:
 - which bonds are polar or non-polar. You will be given a copy (attached to the exam) of the “Summary Chart” - the last page of the “Bio 111 Basic Chemistry Summary” handout (Chemistry 4).
 - any specific chemical structures; you will be given (attached to the exam) a table of amino acid structures listed alphabetically with the exam
 - any specific pathway; if you'll need it, I will provide (attached to the exam) any reactions you'll need
- You may bring in a single sheet of (8 1/2 x 11 inch) paper with any notes you want. You may write on both sides.

Bio 111 Fall 2000 Solutions to Exam II

- 1) a) 231
 b) Many possible answers here:
- remove C-H or C-C
 - add charge or H-bonding capability [remove charge or H-bonding capability]
- For example: (note that, although this structure does not include hydrogen atoms where appropriate, your structure MUST include hydrogen atoms)



- c) Adding another 'philic C=O and O⁻ increase the 'philic properties of the molecule.
 d) See below:



(or to any other lone pair)

- 2) a) i) hydrophobic interaction
 ii) an amino acid with a 'phobic side chain like alanine, phenylalanine, leucine, etc.
 b) i) ionic bond
 ii) an amino acid that has a (+)-charged side chain like histidine, arginine, or lysine
- 3) a) True
 b) true
 c) False: ΔG does not tell you anything about rate so you cannot be sure that this is true; therefore it is false.
 d) False: The ΔG for the reaction $C + ATP \Rightarrow D + ADP + P_i$ is +8, (+20 - 12 = +8) so the reaction is non-spontaneous.
- 4) a) $ADP + P_i \Rightarrow ATP$; $NADH \Rightarrow NAD^+$
 b) $ATP \Rightarrow ADP + P_i$; $NADPH \Rightarrow NADP^+$; $CO_2 \Rightarrow$ glucose

5) NOTE: there will be NO cell biology questions on Exam 2 this Fall.

| | <u>Animal</u> | <u>Plant</u> | <u>Bacterium</u> |
|--------------|---------------|--------------|------------------|
| DNA | has it | has it | has it |
| Protein | has it | has it | has it |
| Lipid | has it | has it | has it |
| Mitochondria | has it | has it | doesn't |
| Chloroplast | doesn't | has it | doesn't |



Bio 111 Handout for Biochemistry 7

This handout contains:

1. Today's iClicker Questions
2. The handout for today's lecture.

iClicker Question #19A - before lecture

Which of the following statements about enzymes are true?

- (A) The substrate binds to the active site.
- (B) The product is released from the enzyme once the reaction is complete.
- (C) The enzyme is unchanged by the overall reaction.
- (D) All of the above.
- (E) None of the above.

iClicker Question #19B - after lecture

Ornithine transcarbamylase disease (OTCD) is an inherited disease where the patient is unable to properly process certain nitrogen-containing waste molecules. As a result of this inability, ammonia (NH_3) rapidly accumulates in the blood of OTCD patients leading to death in infancy.

OTCD is due to the absence of an enzyme called OTC. One copy of a gene encoding functional OTC is sufficient to process all the ammonia produced as a result of a normal diet. The gene for OTC is found on the X-chromosome.

Based on this:

- (A) OTCD would be expected to be inherited in a sex-linked recessive manner.
- (B) OTCD would be expected to be inherited in a sex-linked dominant manner.
- (C) OTCD would be expected to be inherited in a autosomal recessive manner.
- (D) OTCD would be expected to be inherited in a autosomal dominant manner.
- (E) I don't know.

Beaming in your answers

1. Figure out your answer and select the appropriate letter (A-E).
2. Turn on your iClicker by pressing the "ON/OFF" button; the blue "POWER" light should come on. If the red "LOW BATTERY" light comes on, you should replace your batteries soon.
3. Transmit your answer as follows:
 - a. Press the button corresponding to the answer you've selected (A thru E).
 - b. The "STATUS" light will flash green to indicate that your answer has been received. If the "STATUS" light flashed red, your answer was not received; you should re-send it until you get a green "STATUS" light.

Catalase, an enzyme

All of these figures can be viewed using the "Lecture Molecules Application" available for download at the course website.

Catalase enzyme
= 16,000 atoms
= 4 identical protein chains
+ 4 heme groups
• Catalyzes the reaction:
 $2 \text{H}_2\text{O}_2 \rightleftharpoons 2 \text{H}_2\text{O} + \text{O}_2$

Each circle is
an atom

Catalase is made of 4 identical subunits.
• The subunits are deeply inter-twined in the functional enzyme.
• This is unlike hemoglobin where the 4 subunits touch but don't inter-twine.

There are 4 active sites
(the parts where the reaction takes place)
– one in each subunit.

The H_2O_2
(substrate) reaches
the active site via a
tunnel.

The Active site:

These amino acid
side chains hold
the H_2O_2 in
position to react
with the iron atom
in the heme.
The H_2O_2 is held in
place by hydrogen
bonds.

The reaction goes in 2 steps:

(1) the first H_2O_2 goes in:

$\text{H}_2\text{O}_2 + \text{cat} \Rightarrow \text{H}_2\text{O} + \text{cat-O}$
(cat-O = catalase with O bound
to iron in heme)

(2) the second H_2O_2 goes in:

$\text{H}_2\text{O}_2 + \text{cat-O} \Rightarrow \text{H}_2\text{O} + \text{cat} + \text{O}_2$

Notice that the catalase is
returned to its original state.

Bio 111 Handout for Biochemistry 8

This handout contains:

1. Today's iClicker Questions
2. The handout for today's lecture.

iClicker Question #20B - before lecture

Consider Campbell figure 8.9. That figure shows one P-P bond being broken as ATP is converted to ADP + P_i this reaction has a negative ΔG . Which of the following statements is true about this reaction?

- (A) The ΔG is - because a bond is being broken and breaking bonds is always ΔG -.
- (B) The ΔG is - because, although breaking a bond is always ΔG +, not all the bonds are shown and, overall, weaker bonds are being broken while stronger bonds are being made.
- (C) The ΔG is + because bonds are being broken and bond breaking is always ΔG +.
- (D) All of the above.
- (E) None of the above.

iClicker Question #20B - after lecture

Given the following information:



which of the following reactions will occur (be thermodynamically spontaneous)?

(Hint: reversing a reaction switches the sign of ΔG but doesn't change its magnitude)

- (A) glucose + ATP \Rightarrow glucose-6-phosphate + ADP
- (B) glucose-6-phosphate + ADP \Rightarrow glucose + ATP

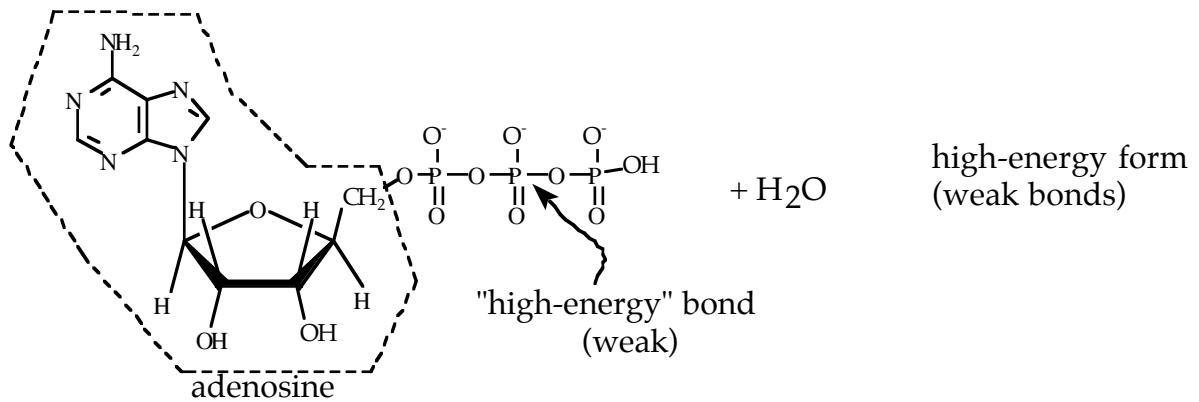
- (A) only A
- (B) only B
- (C) both A and B
- (D) neither A nor B
- (E) I don't know.

Beaming in your answers

1. Figure out your answer and select the appropriate letter (A-E).
2. Turn on your iClicker by pressing the "ON/OFF" button; the blue "POWER" light should come on. If the red "LOW BATTERY" light comes on, you should replace your batteries soon.
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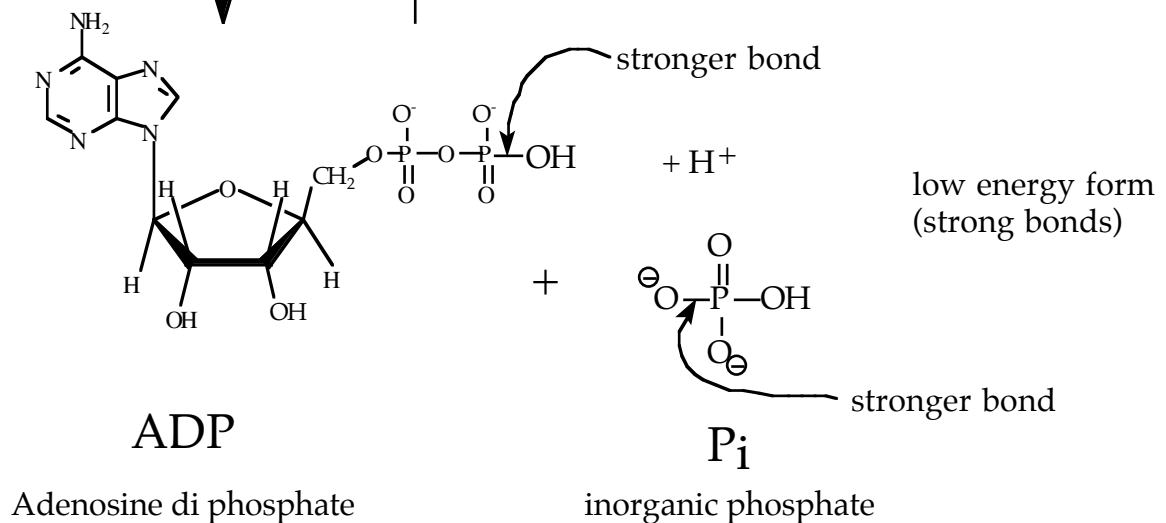
Bio 111 ATP & ADP

ATP Adenosine tri phosphate



releases energy
($\Delta G = -12 \text{ kcal/mol}$)
[spending energy]
{done by many
reactions in cell
needed for life}

requires energy input
($\Delta G = +12 \text{ kcal/mol}$)
[storing energy]
{done by glycolysis/respiration
= "burning food"}



Bio 111 Handout for Biochemistry 9

This handout contains:

1. Today's iClicker Questions

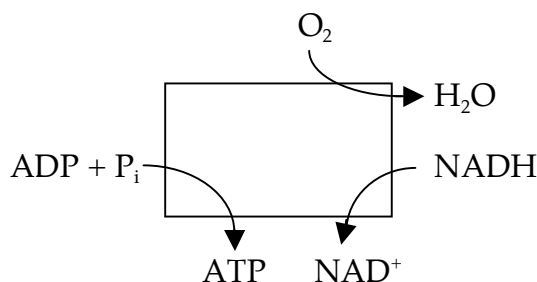
iClicker Question #21A - before lecture

Consider Campbell figure 9.9. In which steps is ADP converted to ATP?

- (A) 1 and 3
- (B) 1 only
- (C) 3 only
- (D) 7 and 10
- (E) 1, 3, 7, and 10

iClicker Question #21B - after lecture

Which process does the diagram below represent?



- (A) the light reactions of photosynthesis
- (B) the dark reactions of photosynthesis
- (C) glycolysis & the Krebs' cycle
- (D) electron transport & oxidative phosphorylation
- (E) I don't know.

Beaming in your answers

1. Figure out your answer and select the appropriate letter (A-E).
2. Turn on your iClicker by pressing the "ON/OFF" button; the blue "POWER" light should come on. If the red "LOW BATTERY" light comes on, you should replace your batteries soon.
3. Transmit your answer as follows:
 - a. Press the button corresponding to the answer you've selected (A thru E).
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