

MMBB 300/380 - Fall 2007
Concepts & Problems - Exam 3

This is intended for your own self testing. Caution, although the following is extensive, do not consider this list to be 100% inclusive for exam purposes; it is possible that some material discussed in class is not covered below.

CATALYSIS

- 1) Compare the binding of substrate, product, and transition state to a typical enzyme. Why are transition state analogs such potent inhibitors? Are they competitive or noncompetitive inhibitors?
- 2) What are catalytic antibodies and what is their association with transition state analogs?
- 3) If given the structure of penicillin, show how it forms a stable (& irreversible) acyl intermediate with glycopeptide transpeptidase. What does this enzyme catalyze?
- 4) How does penicillin interact with β -lactamase? How does this differ from its interaction with glycopeptide transpeptidase?
- 5) Understand the lysozyme catalytic mechanism. What are the two key active site amino acids? Which forms a covalent adduct with part of the substrate? What is the pH dependence of this enzyme? Shouldn't the pK_a of the critical 2 aa side chains be the same? What chemical reaction does lysozyme catalyze? What roles do hydrogen bonds play in the binding of the polysaccharide to lysozyme?
- 6) What are zymogens? Why is it that zymogens can be activated outside of cells?
- 7) In our digestive system, secreted trypsinogen is activated by which proteases? How are secreted chymotrypsinogen, procarboxypeptidases & proelastase activated?
- 8) Describe how preproinsulin becomes mature (biologically active) insulin.
- 9) What is the role of zymogens in the blood clotting cascade? How are the fibrin fibers cross-linked?
- 10) How do hemophilia A & B affect the blood clotting cascade?
- 11) How do coumarin and warfarin act to thin the blood?
- 12) What is the specificity of trypsin, chymotrypsin & elastase? How is this specificity determined?
- 13) Where do these enzymes act on the following peptide? ASDFERTYIKLMNHVCSSGP
- 14) What is the catalytic triad of serine proteases? What is the role of Ser? What is the role of His & Asp?
- 15) Understand the serine protease mechanism including electron movement. At what point and to which atom is the Serine-195 covalently bound? Two products are formed; which leaves first?
- 16) How do the Asp proteases differ from the Ser proteases? How are they the same?
- 17) How has the Asp protease mechanism been exploited to design inhibitors to the HIV protease?
- 18) What are ribozymes? How do they compare to proteinaceous enzymes?
- 19) What simple experiment was carried out by Cech to show that L19 RNA was catalytic?
- 20) What are some of the strategies employed by cells to regulate enzymatic activity?
- 21) How do temperature and pH affect enzyme activity?

MOLECULAR MOTORS

- 1) What are the 3 traditional translocation motor proteins? What cytoskeletal filament does each move along?
- 2) What does it mean to say these motors are unidirectional? What is the difference between the plus & minus ends of actin & microtubules?
- 3) What is the definition of a chemomechanical or mechanochemical enzyme?
- 4) Does kinesin move along microtubules in a hand-over-hand fashion or inch-worm style? What recent experiment proved this?
- 5) In muscle, what are thick and thin filaments? Which of these two is more likely to be considered bipolar?
- 6) Understand the crossbridge model of actomyosin contraction. When is the binding of myosin to actin weak? strong? Which specific event immediately results in the power stroke?

CARBOHYDRATES/METABOLISM/GLYCOLYSIS - Structures

Be Able to Draw the Following Structures (also know the full names)

- 1) ATP, ADP, AMP, Adenosine, NAD^+ , NADH , NADP^+ , & NADPH .
- 2) Glyceraldehyde
- 3) Dihydroxyacetone
- 4) D-Glucose (both open & ring structures & numbering of the carbons)
- 5) D-Fructose (both open & ring structures & numbering of the carbons)
- 6) D-Ribose
- 7) Sucrose
- 8) All glycolytic intermediates (also know all the glycolytic enzymes, which are regulated and know all the small molecules involved with each reaction)

Be Able to Recognize the Identity & Function of the Following Structures

- 1) Creatine, creatine phosphate (phosphocreatine)
- 2) D-Mannose, D-Galactose (open & ring structures)
- 3) Chair & boat forms of sugars
- 4) Lactose & maltose

CARBOHYDRATES

- 1) What is the difference between aldoses & ketoses?
- 2) What are the differences between monosaccharides, disaccharides, oligosaccharides & polysaccharides?
- 3) How many chiral carbons are found in D-glucose? Which carbon is responsible for the D-designation?
- 4) What is the difference between α -D-glucose and β -D-glucose?
- 5) What do the enzymes, maltase, lactase & sucrase catalyze?

METABOLISM OVERVIEW

- 1) What is the difference between catabolic and anabolic pathways.
- 2) Be able to sum up two coupled reactions. For example, couple these two rxns so that PEP converts ADP to ATP; show the net coupled reaction & calculate the change in Gibb's standard free energy:
$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i + \text{H}^+ \quad \Delta G^{\circ'} = -30.5 \text{ kJ/mol}$$
$$\text{PEP} + \text{H}_2\text{O} \rightarrow \text{pyruvate} + \text{P}_i + \text{H}^+ \quad \Delta G^{\circ'} = -62.2 \text{ kJ/mol}$$
- 3) How does the $\Delta G'$ of ATP hydrolysis differ from the $\Delta G^{\circ'}$ of the same reaction?
- 4) What reaction is catalyzed by adenylate kinase?
- 5) What is the typical half-life of a single ATP molecule in a resting cell?
- 6) What is the function of creatine kinase?
- 7) What is NADH ? NAD^+ ? Which form is oxidized? reduced?
- 8) How does NADPH differ from NADH ?
- 9) What does it mean to have a 'high energy' phosphate bond?

GLYCOLYSIS

- 1) What is net yield of glycolysis of a single glucose molecule?
- 2) What is the approximate ΔG of glycolysis in a typical red blood cell?
- 3) Diffusion of glucose across membranes is slow. In vertebrates, how is glucose transported across membranes?
- 4) Which steps of glycolysis are regulated in the typical higher animal? Which effectors regulate these enzymes?
- 5) What is the difference between hexokinase and glucokinase?
- 6) What is the glycolytic commitment step in mammals (and many other organisms)?
- 7) Why aren't red blood cells considered true cells?

LDH

- 1) In very active animal tissues, what is the function of lactate production?
- 2) What is the Cori Cycle?

Partial Key

CATALYSIS

- 1) Why are transition state analogs such potent inhibitors? Are they competitive or noncompetitive inhibitors? **ts analogs tend to be potent competitive inhibitors because enzymes tend to stabilize the ts state of a rxn and thus, enzymes usually bind the ts state (and ts analogs) tighter than either S or P.**
- 4) How does penicillin interact with β -lactamase? How does this differ from its interaction with glycopeptide transpeptidase? **Penicillin forms a covalent bond with an active site serine; this differs from transpeptidase because H₂O is able to attack & hydrolyze the ester bond; with transpeptidase, the penicillin stays bound to the serine.**
- 5) Shouldn't the pKa of the critical 2 aa side chains be the same? **No. The pKa of aa side chains is dependent on the microenvironment created by each protein. In native lysozyme, the pKa for Asp52 is estimated at 3.90 while the pKa of Glu35 is estimated as high as 6.3.**
What roles do hydrogen bonds play in the binding of the polysaccharide to lysozyme? **Lysozyme binds 6 sugar residues simultaneously. Each sugar is very polar and is able to undergo very extensive hydrogen bonding. The enzyme obliges the substrate by forming a large number of these bonds.**
- 6) What are zymogens? **Biologically inactive protein precursors that are activated by proteolysis.**
- 7) In our digestive system, secreted trypsinogen is activated by which proteases? How are secreted chymotrypsinogen, procarboxypeptidases & proelastase activated? **Enterokinase (enteropeptidase) on the mucosal epithelia of the small intestine hydrolyzes trypsinogen to form active trypsin. Trypsin is capable of activating trypsinogen and all the others.**
- 12) What is the specificity of trypsin, chymotrypsin & elastase? How is this specificity determined? **Very close to the active site of the enzyme is a binding pocket that is responsible for binding the amino acid side chain. In trypsin, this pocket is large with a negatively charged Asp at the bottom which stabilizes the binding of Lys & Arg. In chymotrypsin, this pocket is large & hydrophobic to accommodate Phe, Tyr & Trp.**
- 13) Where do these enzymes act on the following peptide? ASDFERTYIKLMNHVCSSGP

Trypsin:	ASDFER	TYIK	LMNHVCSSGP
Chymotrypsin:	ASDF	ERTY	IKLMNHVCSSGP
Elastase:	A	S	DFERTYIKLMNHVC S S GP
- 14) What is the role of Ser? **Attacks carbonyl carbon of the peptide bond & forms ~stable acyl intermediate.**
What is the role of His & Asp? **Provide electron/H⁺ relay which makes the Ser a power nucleophile.**
- 16) How do the Asp proteases differ from the Ser proteases? **One clear difference is that H₂O attacks the carbonyl carbon in the Asp proteases. No stable adduct is formed between enzyme and substrate (like that observed with the serine proteases).**
How are they the same? **They both catalyze the hydrolysis of a peptide bond.**

MOLECULAR MOTORS

- 1) What are the 3 traditional translocating motor proteins? **Myosin, kinesin, dynein.** What cytoskeletal filament does each move along? **Actin (myosin); Microtubules (kinesin & dynein)**
- 2) What does it mean to say these motors are unidirectional? **The filamentous tracks have inherent directionality (i.e. the ends are different). Each motor protein only moves toward one of these two ends.**
When the actin and MT filaments are growing (through polymerization), the actin monomers or tubulin dimers add on faster to the plus end than the minus end; thus, the plus-end is the fast-growing end, while the minus-end is the slow-growing end. A specific molecular motor protein will only move toward the plus-end or the minus-end.
- 3) What is the definition of a chemomechanical or mechanochemical enzyme? **Both names mean the same thing. These are enzymes that convert chemical energy into work or work into chemical energy. Many enzymes fall into this broad definition of a molecular motor.**
- 4) Does kinesin move along microtubules in a hand-over-hand fashion or inch-worm style? What recent experiment proved this? **Hand-over-hand. This was shown by placing a fluorophore on the head or**

motor domain of the kinesin & then measuring how far the kinesin moved the head per step or single ATP hydrolysis.

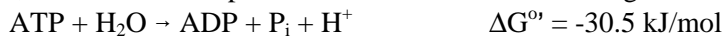
- 5) In muscle, what are thick and thin filaments? Which of these two is more likely to be considered bipolar? **The thick filaments are made of myosin and are bipolar, with myosin heads (motor domains) decorating each end of the filament.**
- 6) Which specific event immediately results in the power stroke? **Release of P_i from myosin. The energy from ATP hydrolysis is stored like a spring in the conformational change in myosin. The release of P_i from myosin triggers the spring to release causing the power stroke.**

CARBOHYDRATES

- 3) How many chiral carbons are found in D-glucose? **Four.** Which carbon is responsible for the D-designation? **Carbon # 5 (the penultimate carbon).**
- 4) What is the difference between α -D-glucose and β -D-glucose? **When the hemiacetal is formed and the closed ring exists, the newly created hydroxyl can either be above the ring, β -configuration, or below the ring, the α -configuration.**
- 5) What do the enzymes, maltase, lactase & sucrase catalyze? **Each of these catalyze the hydrolysis of their respective disaccharide.**

METABOLISM OVERVIEW

- 1) What is the difference between catabolic and anabolic pathways. **Catabolic pathways are degradation pathways that generate energy while anabolic pathways are synthetic pathways that consume energy.**
- 2) Be able to sum up two coupled reactions. For example, couple these two rxns so that PEP converts ADP to ATP; show the net coupled reaction & calculate the change in Gibb's standard free energy:



- 3) How does the $\Delta G'$ of ATP hydrolysis differ from the ΔG° of the same reaction? **$\Delta G'$ is the change in Gibb's free energy accompanying that reaction under those particular conditions (perhaps cellular) while ΔG° is the change in standard Gibb's free energy under standard conditions.**
- 4) What reaction is catalyzed by adenylate kinase? **$\text{ADP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$**
- 5) What is the typical half-life of a single ATP molecule in a resting cell? **Less than 1 minute.**
- 6) What is the function of creatine kinase? **To transfer a high energy phosphate from creatine phosphate to ADP to quickly replenish ATP levels under times of heavy use. $\text{Cr-P} + \text{ADP} \rightleftharpoons \text{creatine} + \text{ATP}$. There is only enough creatine phosphate in muscle to last 5-10 sec of heavy ATP use.**
- 7) What is NADH? NAD^+ ? **Nicotinamide adenine dinucleotide (NAD^+/NADH) is a common redox molecule involved with the oxidation or reduction of a large number of biomolecules. Which form is oxidized? NAD^+ reduced? **NADH****
- 8) How does NADPH differ from NADH? **The 2' alcohol of the adenyl ribose ring is phosphorylated.**
- 9) What does it mean to have a 'high energy' phosphate bond? **This is the exact opposite of high bond energy. A high energy bond means that it is weak, unstable & fairly reactive. The high energy phosphate bonds are compared by how much energy is liberated if the bond is hydrolyzed (cleaved with H_2O). If a high energy phosphate bond liberates more energy than hydrolysis of ATP, then it is possible for that phosphate to be transferred to ADP to make ATP; this happens with the last step of glycolysis where pyruvate kinase transfers a phosphate from PEP to ADP to generate ATP.**
Historically, the cutoff between "high-energy" and "low-energy" bonds is -25 kJ/mol. Below -25 kJ/mol (ie -30, -40, -50 kJ/mol) would be considered high-energy while values greater than -25 kJ/mol (ie -20, -15, -10 kJ/mol) would be considered low energy phosphate bonds. If, by some

chance, the hydrolysis of a specific phosphate bond turns out to be positive, than the reaction would not be favorable in the left-to-right direction.

GLYCOLYSIS

- 1) What is net yield of glycolysis of a single glucose molecule?
Yield = 2 ATP + 2 NADH + 2 H⁺ + 2 pyruvate
- 2) What is the approximate ΔG of glycolysis in a typical red blood cell? **~-100 kJ/mol**
- 3) Diffusion of glucose across membranes is slow. In vertebrates, how is glucose transported across membranes?
Glucose transporters known as the GLUTs, membrane proteins with 12 α -helical amphipathic transmembrane domains are responsible for enhancing the rate of movement 50,000 fold or more over that of diffusion.
- 5) What is the difference between hexokinase and glucokinase? **Hexokinase is present in most cells of most organisms and is fairly nonspecific (phosphorylates different monosaccharides) but with low K_M . Glucokinase is specific to higher animal liver and is very specific but with a high K_M (only works well when glucose levels are high).**
- 6) What is the glycolytic commitment step in mammals (and many other organisms)? **The conversion of Frc-6-P to Frc-1,6-BP by phosphofructokinase-1 (PFK-1).**
- 7) Why aren't red blood cells considered true cells? **No nuclei; no mitochondria. Lacking most organelles, the red blood cell (erythrocyte) could be considered a biochemical bag that is rich in hemoglobin.**

LDH & Gluconeogenesis

- 1) In very active animal tissues, what is the function of lactate production? **Consume NADH & H⁺ and regenerate NAD⁺ so that glycolysis can continue.**
- 2) What is the Cori Cycle? **During strenuous activity, lactate produced by muscle travels to the liver (via the blood) where it undergoes gluconeogenesis to resynthesize glucose which is dumped back into the blood to supply the muscle (and other hungry tissues).**