

This is intended for your own self testing. Caution, do not consider this list to be 100% inclusive for exam purposes. For instance, this list does not cover specific topics from the 380 extra hour.

Protein Structure (Lecture 6)

- 1) List the 4 levels of protein structure; compare & contrast.
- 2) Compare & contrast the α -helix & β -pleated sheet structures. Where are the hydrogen bonds? Where do the side chains of the amino acids project? What role do the side chains play in forming α -helix & β -pleated strands?
- 3) Describe a β -turn. Where are they found?
- 4) What is an α -helical coiled-coil?
- 5) Ribbon diagrams of 3D protein structure use helices & flattened arrows; what do these depict?
- 6) What is a protein domain?

Myoglobin & Hemoglobin (Lectures 7 & 8)

- 1) What is a prosthetic group?
- 2) What is heme & how does it bind to Fe^{2+} ?
- 3) What are the distal & proximal Histidines in these 2 proteins? What is their role in O_2 binding?
- 4) Sequence comparisons of hemoglobins from various species show only 9 aa residues that are strongly conserved; yet their 3-D structures are virtually identical. What is the significance of this? Are the distal & proximal His conserved?
- 5) What were the contributions of John Kendrew & Max Perutz? What were some important features of these structures?
- 6) Why is hemoglobin an allosteric protein? What does it mean to bind O_2 cooperatively? Does myoglobin exhibit allosteric behavior?
- 7) What is the Bohr Effect? Is this effect the result of an allosteric interaction? How do CO_2 & H^+ interact with the hemoglobin subunits?
- 8) Know how to read an O_2 saturation curve (saturation vs $p\text{O}_2$). What does $p\text{O}_2$ mean?
- 9) How does 2,3-Bisphosphoglycerate affect O_2 binding in hemoglobin? How is 2,3-BPG associated with high altitude & mother-fetus O_2 exchange?
- 10) What is the molecular explanation of sickle cell anemia? What is the precise difference at the protein level? Under what conditions does the HbS polymerize and why? What is meant by a 'molecular disease'? How does sickle cell anemia confer resistance to malaria?

Special Topics on Protein Structure (Lecture 9)

- 1) What is unusual about the structure of collagen helix? What is the role of Gly in this structure?
- 2) What post-translational modification of collagen requires Vitamin C? What is scurvy?
- 3) What is the difference between procollagen & tropocollagen?
- 4) How is β -sheet structure involved in the structure of silk?
- 5) Describe chemical & biological differences between the normal prion protein, PrP^c , & the infectious prion protein, PrP^{Sc} .
- 6) Once an infectious prion protein, PrP^{Sc} , is present in neuronal tissue, how is more PrP^{Sc} formed?
- 7) For variant Creutzfeldt-Jacob Disease (vCJD), codon 129 of the PrP^c gene is significant. Explain.

Free Energy & Thermodynamics (Lecture 10)

- 1) Considering a chemical reaction, what do thermodynamics & kinetics allow us to predict?
- 2) What are ΔG , ΔG° , $\Delta G'$, $\Delta G'^\circ$? What are standard conditions?
- 3) What is the energy of activation & how does it affect the kinetics & thermodynamics of a reaction?
- 4) The fastest enzymes turn over at what rate?
- 5) What does $\Delta G > 0$, $\Delta G = 0$, & $\Delta G < 0$ tell us about the favorability of a given reaction?
- 6) How do catalysts/enzymes affect ΔG or $\Delta G'^\circ$? How do catalysts affect the energy of activation? Be able to show this using a free energy diagram.

Introduction to Enzymes (Lecture 10)

- 1) What is an enzyme?
- 2) Enzyme active sites share what common features?
- 3) Know the difference between the 'Lock and Key' model and Koshland's 'Induced Fit' model.
- 4) Know the 5 important properties of enzymes.
- 5) Know the 6 major classes of enzymes: both names & type of reaction.

Enzyme Kinetics (Lectures 11, 12 & 13)

- 1) How does an enzyme affect the rate (kinetics) & the equilibrium (thermodynamics) of a rxn?
- 2) Know how the Michaelis-Menten eqn describes an enzyme-catalyzed rxn. Know the eqn & what it means.
- 3) What is K_M ? Is it a binding constant?
- 4) What's special about a) $[S] \ll K_M$; b) $[S] = K_M$; & $[S] \gg K_M$
What can we say about the initial rate (v) of the reaction under these conditions?
- 5) What is the Lineweaver-Burk reciprocal plot? What information do we get from this plot? Be able to sketch or identify all of these on a Lineweaver-Burk plot.
- 6) Compare & contrast competitive and noncompetitive inhibition. What plot do we use to distinguish these? How are V_{max} & K_M affected?
- 7) What are mixed mode & uncompetitive inhibition & how are K_M & V_{max} affected?
- 8) How do reversible inhibitors compare with irreversible inhibition?
- 9) What is the v/V_{max} ratio when a) $[S] = 0$; b) $[S] = 1/10 K_M$; c) $[S] = K_M$; d) $[S] = 10 K_M$

Solutions to selected problems follow on the next page.

Selected Solutions

Protein Structure

- 1) **Primary = Linear sequence of amino acids; Secondary = Local structure of amino acids relative to each other, includes α -helix, β -sheet, & β -turns; Tertiary = 3D structure of a protein or one of its domains; Quarternary = Structure of a protein complex that has more than one subunit.**
- 2) **Since side chains stick out away from the α -helix & β -pleated sheet, the aa side chains only play a small role in stabilizing the structure. The structure is stabilized primarily by hydrogen bonds between polypeptide backbone carbonyl oxygens and amide nitrogens.**
- 5) **helices = α -helix; one flattened arrow = a single β -strand**
- 6) **Protein domains are distinct, usually globular units of a protein. The titin example given in lecture is an excellent example of a single polypeptide (albeit a huge polypeptide) that has multiple globular domains linked by flexible stretches of the polypeptide.**

Myoglobin & Hemoglobin

- 1) **A prosthetic group is an organic, nonpolypeptide unit (which may be more than one molecule) which must bind the protein for normal activity.**
- 3) **The proximal His is bound to Fe^{2+} ; the distal His forces CO to bind with a bent, weaker geometry = weakens CO binding; the distal His also H-bonds with both molecular oxygen, O_2 & CO.**
- 4) **The distal & proximal His are conserved in these proteins. The reason that most of the aa are conserved is because most are not directly involved with binding Heme and O_2 . It is possible to mutate many of the amino acids to another amino acid without changing the function of the protein or the overall structure of the protein. It also means that different amino acid sequences can give rise to essentially the same tertiary structure.**
- 6&7) **Hemoglobin demonstrates several allosteric interactions which means that the binding of a molecule at one site of a protein affects the activity or binding at a distant, second site on the protein; typically this occurs by altering the 3D/tertiary structure of the protein. For example, binding of O_2 in one subunit of hemoglobin enhances the binding of O_2 in the other 3 subunits. The binding of either H^+ or CO_2 decreases hemoglobin's affinity for O_2 (the Bohr effect). The H^+ is picked up by the C-terminal His on each of the β -subunits while the CO_2 carbamylates the NH_2 -termini of both α - & β -subunits.**
- 9) **2,3-BPG binds between the 2 β -subunits of adult hemoglobin ($\alpha_2\beta_2$); this favors the T state of Hb and weakens the affinity for O_2 . Fetal Hb ($\alpha_2\gamma_2$) does not bind 2,3-BPG which favors exchange of O_2 from the mother Hb to the fetal Hb. At higher altitudes, 2,3-BPG levels in the blood go up to assist in greater release of O_2 in the tissues.**
- 10) **A single aa change in the β -subunit of Hb alters Glu_6 to Val_6 . This changes a charged, acidic amino acid to a hydrophobic aa. This new valine puts a hydrophobic aa side chain right at the surface of the hemoglobin protein. When the sickle cell Hb(HbS) is deoxygenated, the structure is such that two HbS bind a hydrophobic patch on each other. More HbS proteins add on to create a large, stable filament that eventually fills the cell and causes the cell to form a sickle shape. In malaria, the pH inside infected drops which favors the deoxygenated form of HbS which favors polymerization/aggregation of the HbS which causes infected cells to be destroyed quickly; this helps keep the infection in check.**

Special Topics on Protein Structure

- 1) **Collagen is a triple helix where the side chain of every 3rd amino acid sticks into the center of the helix. This why every third aa is glycine (no side chain).**
- 2) **Vitamin C is required for the oxidative hydroxylation of Pro (& Lys). The hydroxy-prolines allow additional H-bonding which strengthens the collagen structure. Without the added hydroxyls, collagen is weaker & connective tissue begins to be less connective & lesions of the skin, gums and loss of hair are included as symptoms of scurvy.**

- 4) The β -strands form β -sheets like normal but then the sheets are stacked on top of each other to make a box-like structure which is very strong. In this sort of stacking, the side chains play an important role; Gly & Ala are common amino acids in these proteins. The β -boxes or β -cubes are held together by α -helices which provide silk with its elasticity.
- 5) If PrP^C & PrP^{Sc} are from the same organism, the primary aa sequence may be identical. The secondary & tertiary structures can be very different: PrP^C contains 4 α -helices while one form of PrP^{Sc} has 2 α -helices and 4 β -strands; different forms of PrP^{Sc} might have different 3D structures.
- 6) The infectious prion protein, PrP^{Sc} can be formed when PrP^C binds to the infectious form. The infectious form polymerizes to form very stable filaments that the cell is unable to degrade. The exact mechanism is not known. Eventually these filaments or aggregates break open the brain/nerve cells; this leads to spongiform encephalopathy.
- 7) 100% of all individuals that have contracted vCJD are homozygous for Met at codon 129 of the PrP^C gene. This is a significant finding because only ~42% of the UK population is homozygous for Met. In addition, ~90% of all individuals that come down with CJD (~1 in a million each year) are homozygous for either Met or Val; the reason for heterozygotic resistance is unknown.

Free Energy & Thermodynamics

- 2) The prime in $\Delta G'$ & $\Delta G^{o'}$ means that the solution is at pH 7.0.
Standard conditions are defined as: [S] = [P] = 1.0 M, 1.0 atm, 25°C.
- 4) Catalase has the fastest turnover (k_{cat}) at 40 million sec^{-1} ($4 \times 10^7 \text{ sec}^{-1}$)
- 5) When $\Delta G > 0$, the rxn is favored right-to-left (the “reverse” direction); When $\Delta G = 0$, the rxn is at equilibrium; When $\Delta G < 0$ the rxn is favored left-to-right (the “forward” direction).
- 6) Catalysts/enzymes do not alter ΔG or $\Delta G^{o'}$. Catalysts lower the energy of activation.

Introduction to Enzymes

Enzyme Kinetics

- 3) K_M is a ratio of rate constants that gives us an indication of how tightly the substrate, S, is bound to the enzyme. K_M is also the substrate concentration, [S], at which the enzyme is working at $1/2$ the maximum rate. The lower the value of K_M , the stronger the binding.
- 4) When $[S] \ll K_M$ then $v = V_{max} [S]/K_M$ the rate of the reaction depends linearly on substrate conc.
When $[S] = K_M$; the rate is at $1/2$ the maximal rate.
When $[S] \gg K_M$ the rate is maxed out ($v = V_{max}$)
- 5) The Lineweaver-Burk reciprocal plot is used to get values for V_{max} and K_M and it is used to characterize the different types of inhibitors.
- 6&7) There are 4 common types of enzymatic inhibition that can be identified using the Lineweaver-Burk double reciprocal plots. Competitive, noncompetitive, uncompetitive & mixed. In all cases, V_{max} or K_M or both are altered by the factor $(1 + [I]/K_i)$.
- 9) $v/V_{max} = [S]/(K_M + [S])$; a) $v/V_{max} = 0/(K_M + 0) = 0$;
b) $v/V_{max} = 1/10 K_M/(K_M + 1/10 K_M) = 1/11 = 0.091$
c) $v/V_{max} = 1/2$
d) $v/V_{max} = 10 K_M/(K_M + 10 K_M) = 10/11 = 0.91$