CHAPTER 5, appendix
1. Please read the Appendix to Chapter 5. Proteins can be separated according to their size, their charge, or their affinity. Gel Filtration, Dialysis, and Ultrafiltration are "size only" methods. Gel Filtration can be used to calculate a fairly accurate molecular weight if the protein involved is spherical. Log MW is plotted against retention time (the graph resembles the one shown in Figure 5A.4, p. 155) and the protein is compared to spherical standard proteins.

2. There are various forms of Electrophoresis. "Classical" Electrophoresis separates on the basis of both size and native charge. SDS PAGE separates on the basis of size only, after denaturation. Isoelectric Focusing separates on the basis of charge only. SDS Page and Isoelectric Focusing are combined in 2-Dimensional Gel Electrophoresis, a powerful method of protein separation.

3. Affinity Chromatography can't be used on every protein, but if a desired protein has a known affinity it can greatly simplify and speed the purification process. The disadvantages are expense and sometimes inconvenience, if you have to make your own beads.

4. Ultracentrifugation can reveal a lot about a protein. Equilibrium Sedimentation gives a good molecular weight value for an intact protein (no denaturation required). Rate Sedimentation is a "quick and dirty" method which gives information about the combined size and shape of the material. Information from Rate Sedimentation is expressed in Svedbergs, and a good example is ribosomal subunits (remember 30S + 50S = 70S).

CHAPTER 15
1. Understand the kinds of enzyme control summarized on page 462-3: induction-repression (synthesis control), covalent modification (action at a distance), and allosteric regulation (fast response in the cell). These will be covered in more detail within the discussion of metabolism. Zymogen cleavage is specifically for protease (protein cutting) enzymes.

2. Read through section 15.5 (pages 480-492). The highlights of this material will be covered in class. The single most important fact in the section is that Myoglobin is a monomer, whereas Hemoglobin is a tetramer. That allows the two molecules to bind Oxygen in different ways so that Hemoglobin can deliver it from lung to capillary in an efficient manner.

3. Structurally, Myoglobin and the α or β subunits of Hemoglobin are quite similar (look at Fig 15.28, p. 483). Both follow the "globin fold" which is mostly alpha-helical, and binds to a heme group. The heme group (a prosthetic group) contains an iron atom, and that is what interacts directly with the oxygen. Thus, Myoglobin binds a single molecule of oxygen and Hemoglobin binds four (Fig 15.23).

4. The functionality of the system is best seen in a loading curve (Fig 15.22). Since Hb is an allosteric protein, its binding curve is sigmoidal, where Mb has a hyperbolic curve. The distance between the curves shows how much oxygen can be transferred at a given ambient O₂ level. You should know that normally Hb functions in the presence of 2.3 BPG (p. 490) which favors the deoxy state.