BERG/STRYER V STUDY GUIDE

CHAPTER 4 rev2

1. HOMEWORK 1-3, 5, 7, 10. Answers on p. C-2, solutions in *COMPANION*. Figs 4.2, 4.3, 4.4, 4.5, 4.7, 4.10. Correct Fig 4.11 switch "High" and "Low." Proteins can be purified using techniques that involve electrophoresis, chromatography, ultracentrifugation, and other methods. You should understand the difference between **electrophoresis** with and without SDS, and know what **SDS PAGE** stands for. SDS PAGE is frequently used to obtain a quick estimate of a protein's molecular weight. What is **isoelectric focusing**? Dialysis and gel filtration are separations on the basis of size. **Gel filtration** can produce good separations and can be used to estimate molecular weight if the protein is globular. **Affinity chromatography** can be an extremely effective way to purify certain proteins. Ion exchange is more useful with amino acids and peptides than with proteins, and gives a rather crude separation.

2. There are several different modes of **ultracentrifugation**, used for different purposes. **Rate** sedimentation (p. 88) gives a "quick and dirty" idea of the size of a protein or complex, in Svedberg units (S). **Zonal** centrifugation (Fig 4.15, p. 89) is a preparative technique. **Sedimentation Equilibrium** is a time consuming and rigorous method for determining the molecular weight of a protein in non-denaturing conditions. This is important since most other molecular weight methods require that the protein be denatured.

3. Once a protein has been purified, there are numerous methods for determining its properties. And extremely accurate molecular weight can be obtained by the new technique of **MALDI-TOF spectroscopy** (p. 90). **Amino acid analysis** (91) will tell which amino acids are present (but not their sequence). Know the structure and reaction of Ninhydrin and understand the pH changes in Fig 4.18. End group labeling reagents can identify the first amino acid in the chain – Fluorescamine, Dabsyl chloride, Dansyl chloride, and FDNB or Sanger's Reagent are variations on this theme. The Edman Degradation both labels and removes the first amino acid, so that it can be used for **sequencing**. Know the structure and reactions of Edman's reagent, phenyl isothiocyanate (Fig. 4.21). Sequencing a single sequence of 300 amino acids is difficult -- the problem can be simplified by cutting the chain into smaller pieces. Know where Trypsin, Chymotrypsin, and Cyanogen Bromide cleave peptide chains. Performic acid, BME, or Dithiothreitol can be used to separate disulfide crosslinks. Understand that the most modern techniques involve finding and sequencing the **DNA** in the gene for a protein, and then deducing the amino acid sequence from the DNA sequence. This is described on 98-99. What *use* is a protein sequence? As yet, we can not deduce the 3-D folding of proteins from their sequence. The best uses are taxonomic (determining relationships between species) and evolutionary (relationships between proteins). Read discussion 96-97.

4. There are some powerful techniques based on manipulation of **antibodies** (98 ff.). Monoclonal antibodies are a population of identical antibody molecules, all directed at one part of a protein (epitope). An important use of these is in **ELISA**, enzyme linked immunosorbent assay (Fig 4.35). The most familiar example of ELISA is the home pregnancy test, which "looks" for hCG or human chorionic gonadotropin. hCG is a small acidic protein that escapes into the urine during pregnancy, and it is fairly easy to detect. http://www.whfreeman.com/biochem5 has a nice demonstration of Elisa for detecting hCG (and much other useful information!). **Western blotting** (Fig. 4.36) is a related technique used in the laboratory.

5. You should understand *generally* how **Solid-phase Synthesis** of peptides works (104 ff.) but you don't have to memorize the structures of the blocking and activating groups. Solid phase synthesis, in which the growing chains are attached to resin beads, allow easy removal of byproducts and spent reagents, and result in peptides of high purity and high yield.

6. It is important to realize that, at present, the only ways to determine the 3-D structure of a protein are **X-ray crystallography** and **NMR spectroscopy**. It is usually rather difficult to crystallize proteins, so many more sequences are known than structures of proteins. Known sequences and structures are uploaded to databases for worldwide use. One copy of this database is at Rutgers: http://rutgers.rcsb.org/pdb/.