BioChem 3070 - Review of Concepts for First Hour Exam

2 **Protein Composition and Structure**

Amino acid structures and properties; acid/base reactions, organic functional groups
Draw chemical structures of all 20 amino acids and polypeptides at specified pH values.
Amphoteric nature of amino acids, zwitter ions, electrophoretic mobility
Primary structure - peptide bonds, linear sequence; be able to draw short peptides
Secondary structure – *alpha*-helix, *beta*-sheet, Van-der-Waals interactions (H-bonds) that stabilize
Tertiary structure - Overall shape, Anfinson's work & -S-S- bonds
Quaternary structure - Multiple protein subunit interactions

3 **Exploring Proteins**

Isolation & purification of proteins: Assay, solubility, size, charge, affinity
Differential centrifugation, salting out proteins, dialysis
Chromatography: gel fitration, ion exchange, affinity & Electrophoresis
Characterization of proteins:

SDS/PAGE, ultra-centrifugation (S-values), zonal centrifugation, amino acid composition, X-ray crystallography, "NOESY" NMR, Edman (automated) degradation & sequencing, peptide cutting tools (CNBr, trypsin, carboxypeptidase), Western Blots, ELIZA

4 **Nucleic Acids: DNA, RNA and the Flow of Genetic Information**

DNA - composition, structure, dimensions, Watson and Crick's original publication
"Melting" DNA, T_m, "annealing" DNA, compositional effects on T_m
Chromatin structure and composition: histones, nucleosomes, linker strands
DNA replication; semi-conservative, templates, ^{15}N-DNA experiments demonstrating conservative replication
Kornberg's DNA polymerase, primers, ligase, direction of polymerization, nucleophilic attack of 3'-OH
DNA mutations: nitrous acid, intercalation dyes, UV light, substitution vs. frame shift, repairs
Identifying mutagenic chemicals: Ames test
RNA: transcription and translation; sites for both, process for each.
Various forms of RNA: messenger, transfer, ribosomal
RNA processing (introns & exons) ribosomal assembly, translation, sites for antibiotic action
Translation: ribosomal assembly, translation, peptide bond formation, sites for antibiotic action
Genetic code: nature and translation, major features; degeneracy, ramifications of mutations

5 **Exploring Genes & Genomes**

Restriction Enzymes; palindrome specificities, sticky ends, applications
Sequencing DNA in gels & capillary electrophoresis, Sanger dideoxynucleotides method
DNA probes and synthetic DNA; hybridization, Southern & Northern Blots
PCR reaction: Kerry Mullis, components, process, thermocycling, results
Recombinant DNA: vectors, plasmids, restriction enzymes, ligase, methods of transfection
Reverse transcriptase and its role in genetic engineering, manipulating & transforming cells

7 **Oxygen Transport Proteins – Myoglobin and Hemoglobin**

General structure of myoglobin: protein, heme, iron, chelation of hexadentate ferrous/ferric ion
Myoglobin stores O_2 in the tissues, but is not present in the blood
Hemoglobin (Hb) is composed of 4 myoglobin-like subunits: 4 proteins, 4 protoporphyrins, 4 iron atoms,
Adult Hb contains two identical pairs of subunits, $\alpha_2\beta_2$ that bind O_2 cooperatively, with a sigmoidal O_2 binding curve
2,3-BPG binds to a center cavity of deoxyhemoglobin, drawn by positively charged groups on the β-chains.
Protein subunit interactions, 2,3-BPG concentration, an pH all affect the cooperative binding of Hb to O_2.
Histidine blocks the linear bonding of O_2 and other more polar gasses, facilitating their release
Hb's sigmoidal binding curve is defined by the mathematical model: $Y = pO_2^n / (pO_2^n + p50^n)$.
Calculate Y for one or two sets of pO_2 and $p50$ values, and report ΔY.
The Bohr effect is essentially the effect of lower pH promoting the release of oxygen from Hb.
CO_2 is also transported by Hb, however not at the heme, but as carbamates with the terminal amino groups
Sickle-cell Anemia is caused by a substitution of valine for glutamate in the β-chain of Hb. *(Error in 7ed Textbook!)*
These valine residues provide a non-polar sticky site that promotes the polymerization of de-oxy Hb molecules.