Biochemistry I - Final Exam Review Guide. ~1/2 Nucleic Acids + ~1/2 Previous Material
Review Problem sets and recitations first, then major concepts in the lecture note, then OLI.

DNA Structure: You should be familiar with the structure of nucleic acids:
-polyanion, **deoxy**ribose, 5' end and 3' end
-glycosidic bond & how the bases are connected to the ribose
-anti-parallel nature of double stranded DNA, topology, supercoiling, major and minor groove.
-syn anti orientation of bases, exo endo conformation of sugar.

RNA Structure: How it is different from DNA
2' OH group, T base replaced by U. Easily hydrolyzed under alkaline conditions.

Forces that stabilize nucleic acid & how they differ from protein stability, bilayer stability, etc.

Bases: Be able to distinguish between purine/pyrimidine Max. absorbance of UV light occurs at 260 nm

Base pairs: A-T, G-C, Wobble base pairing in codon-anticodon interaction
Come prepared to identify the major groove, minor groove, and hydrogen bonding groups, *both* those involved in Watson-Crick Base pairing as well as those involved in protein-DNA interactions.

Cloning & Expression:

- Be familiar with the overall scheme, i.e. conversion of HIV viral RNA to dsDNA, PCR method and primer design, construction of an expression plasmid (or vector), DNA sequencing method (see below). Use of restriction digests and gel electrophoresis to confirm the correct plasmid.
- Be familiar with how protein production is controlled using inducible expression systems.
- Be able to correctly design a PCR product that can be inserted into T7 vectors to produce protein.
- Protein export using leader or signal peptide, purification by nickel affinity chromatography.

Some details regarding the 'tools'

- Why do plasmids have origins of replication & genes that encode proteins that confer antibiotic resistance?
- How are restriction endonucleases and DNA ligase used in the construction of plasmids?
- How to measure DNA fragment sizes by gel electrophoresis, use this can be used to test whether a plasmid has been constructed correctly.

DNA Protein Interactions:

- Non-specific recognition of phosphates using Lys and Arg.
- Recognition of specific DNA sequences by hydrogen bonding, typically in the **major** groove.
- Be prepared to make predictions regarding how much protein is bound to DNA, given a K_D. Come prepared to determine how changes in the protein or DNA will affect binding.
- Methods to measure DNA binding, how to interpret gel-shift assays to determine K_D.

mRNA synthesis, Protein Synthesis: These are both examples of polymer formation using a template. Before studying the details of each of these you should look for the following generalities (i.e. make a chart of the following format. I have filled in a few entries - try filling out the rest!

Pathway	**Template**	**Monomer Substrate**	**Product**
DNA transcription (mRNA synthesis)		NTPs	
Translation (protein synthesis)			Proteins

Next, concentrate on the following general steps in each pathway: initiation, propagation, and termination. Include **sites** on nucleic acid and **factors** that bind to them. (Again, I have filled in some of the blocks):

Pathway	**Initiation**	**Propagation**	**Termination**
DNA transcription (mRNA synthesis)		Core enzyme works until stop signal is reached.	
Translation (protein synthesis)			Stop codon, termination factors.

After you have filled in the above, you should focus on the following details:

DNA Polymerases:

- Primer required, providing 3'-OH group.
- Polymerization in 5'->3' direction, fidelity by WC H-bonds & pyrimidine-purine size matching.
- Polymerization driven by indirect coupling by hydrolysis of pyrophosphate.
- Role of 3'-5' exonuclease in proofreading.
- Differences between HIV reverse transcriptase and other polymerases (ie. proofreading activities)

DNA Sequencing

- Come prepared to explain how the data is generated.
- Convert to protein sequence with codon table (including finding the reading frame).

PCR:

- Primer design in terms of T_M, 3'-GC clamp, primer dimers, addition of restriction sites.

HIV Reverse transcriptase:

- What does this enzyme do in the life cycle of the HIVs virus?
- How does its property affect the fidelity of replication and drug resistance?

DNA Transcription (mRNA synthesis)

- Features of RNA polymerase (core/holo, 5' to 3' template directed synthesis)
- Features of promoter (-35 and -10 regions) and termination signals.
- Repression of mRNA production by binding of a repressor to operator sequences.

Protein Synthesis (RNA translation)

- Genetic code - triplet of bases=1 amino acid, start and stop codons, wobble basepairing.
- tRNA structure - general features of tertiary structure, anti-codon loop & 3'CAA acceptor stem.
- Charging of tRNAs is catalyzed by aminoacyl-tRNA synthetases. Editing of mischarging.

Ribosomes:

- RNA + protein, RNA provides catalytic groups. Two subunits (30S+50S)
- mRNA binds to the 30S subunit, positioned by the interaction of the SD sequence and the 16S RNA
- Contain three sites: **E**- Exit site for uncharged tRNA, **P**- site for Peptidyl-tRNA, **A**- site for next charged Amino-acyl-tRNA.
- Both the 30S and the 50S contribute to the P and A site, only the 50S contributes to the E site.
- Chain initiated by formation of complex of mRNA, fMet-tRNA, 30S, 50S, fMet-tRNA in the P-site.
- Incoming charged tRNAs move in the following direction: **A→P→E.**
- Termination is by recognition of stop codon by termination factors.

Special Topics (if discussed): Site-directed mutagenesis, expansion of genetic code.

Key topics from the early part of the course:

- Amino acid structure and properties (UV absorbance, pK_a of sidechains, charge calculations)
- Buffers – concept of buffers & how to construct a simple buffer.
- Acid/base effects on: Enzyme activity, ligand binding, protein purification, etc.
- Properties of peptide bond. Planer/cis/trans
- Proteins: features of 2^o, 3^o, 4^o structure, Ramachandran plot.
- Fundamental thermodynamic forces and how they affect structure & function of biological molecules (proteins, membranes, membrane proteins, DNA)
- hydrophobic effect & conformational entropy (ΔS). Role in protein folding, membrane assembly.
- van der Waals, H-bonds, electrostatics (ΔH)
- Thermodynamics:ΔG, ΔG^o, ΔH, ΔS, K_{EQ}
- Ligand binding: Data analysis, evaluation of cooperativity, relationship between K_D, k_{off} and molecular interactions. Hill coefficient and cooperativity.
- Oxygen transport
- Antibodies: Overall structure and functional properties.
- Enzymes: Fundamentals of rate enhancement and substrate specificity. Role of functional groups in substrate specificity and catalysis.
- Enzyme kinetics: Determining K_M, V_M, K_I, K_I' & *significance* of these parameters.
- Allosteric control of enzymes (metabolism, lac repressor) & allosteric effects in hemoglobin
- Protein purification & molecular weight determination (SDS-gel/gel filtration)
- X-ray diffraction in structure determination.
- Metabolic pathways: glycolysis/gluconeogenesis/anerobic/TCA/e-transport/fatty acid oxidation, ATP generation.
- Regulation of metabolic pathways, general approach plus some examples, i.e. hormonal control, F26P & PFK, PFK and energy sensing. Carbohydrates: Know the structure of glucose, ribose, and fructose. In addition, general structure plus some features in energy storage and structure.
- Lipids: Know how to draw a phospholipid. Relate structure to features of biological membranes (T_M, permeability, membrane protein structure – e.g. ion channels). Relationship between CMC and polarity of lipid.
- Carbohydrates: Aldose versus ketose, ring formation, disaccharide formation, polysaccharides.