Review Summary – CH370 - Exam 1

Amino Acids and Peptides

Know all 20 common amino acids – name / 3-letter abbrev. / 1-letter abbrev.

Know approximate pKa's of titratable amino acids (2/4/6/8/10/12)

Charge properties of amino acids and peptides / pI

Nature of the peptide bond (phi / psi angles)

Protein Structure

Definitions of primary, secondary, tertiary and quaternary structures

Common secondary structures / Phi, Psi (ϕ/ψ) torsion angles

How to read a Ramachandran Plot

Common terms used to describe protein structure – motifs / domains - examples

Review of Nucleic Acids: Structures / Folding

Know N Bases; Primary & Secondary structure: double helix by Watson & Crick -1953

Nucleotide pairings: Watson-Crick

Conformations of nucleosides - syn / anti; Sugar pucker: endo or exo

Stabilization (destabilization) Hydrogen Bonding / Electrostatics / Stacking

Denatured DNA: Heat denaturation of DNA is called "melting," Tm / hypochromism.

DNA Sequencing – Maxam-Gilbert vs. Sanger (basics; how to read a sequencing gel)

Bioinformatics and Software

Major web resource sites - NCBI / EMBL / ExPASy / PDB

BLAST – principles, uses and definitions of **key terms**,

Substitution matrices

Sequence alignments / Scoring

Protein Purification

Produce / Extract / Purify

Produce: rich tissue / expression system

Extract: cell lysis – grinding / sonication / French Press / detergent

Purify: Take advantages of differences in: Solubility / Charge / Size / Specificity

- various forms of chromatography

Analysis: Follow purification using an assay for "activity" and SDS gels

Spectroscopy

Interaction of Light with Matter (induce oscillating dipoles in matter)

a) Scattered –
$$(\sim 10^{-16} \text{ sec})$$
 b) Absorption - $(\sim 10^{-15} \text{ sec})$

Absorption Spectrum – "fingerprint"

Beer-Lambert Law: Absorbance (A); Intensity (I, I_0); Transmittance (T = I / I_0)

 $A = \log (I_0 / I) = \log (1/T)$

Extinction Coefficient – E (1%), ε_{M} = Molar extinction coeff.

$$A = O.D. = \varepsilon \bullet c \bullet l$$
 also $[E^{1\%}] \bullet MW = 10 \bullet [\varepsilon_M]$

Fluorescence / Phosphorescence

Fluorescence ($\sim 10^{-4}$ sec to 10^{-9} sec) / Phosphorescence ($> 10^{-3}$ sec)

FRET (Fluor. Res. Energy Transfer) Eff. = $1/[1 + (R/Ro)^6]$ - needs "spectral overlap"