Basic Chemistry

- Identify chiral centers on molecules, and discuss implications of chiral centers on drug activity.
- Identify hydrogen bond donors and acceptors on proteins, DNA, drugs, etc. (X-H Y, X and Y are electronegative, e.g. O and N).
- Effect of charge on ability of drug to cross membrane.
- Understand the fundamental molecular basis for:
  i) electrostatic interactions
  ii) van der Waals interactions
  iii) hydrophobic effect.

Protein structure – important characteristics of each level:

1. Primary structure:
   - Know how amino acids are linked together by peptide bonds to form proteins, given the structure of two amino acids, could you draw the correct structure after they are joined?
   - Nomenclature – sequence begins at the amino terminus.
   - Know that order (sequence) of amino acids defines folded structure, defines activity.
2. Secondary structure - conformation of the mainchain
   - two that can be stabilized by hydrogen bonds are α-helix and β-sheet.
   - know structural properties (location of H-bonds, sidechains)
3. Tertiary structure
   - Relate structure to energetic terms:
     - Folded form stabilized by burial of hydrophobic groups, therefore core is composed of non-polar amino acids – hydrophobic effect.
     - Folded form stabilized by van der Waals interaction in the core – therefore core is well packed.
4. Quaternary Structure
   - Structure with multiple chains, e.g. antibodies

Enzymes

- Catalysts – make reactions go faster without being changed by the reaction.
- Active site – part of the enzyme that has amino acid residues that recognize substrate and facilitate chemistry on the substrate, converting it to product.
- Increase in rate occurs because enzyme decrease the energy of the high energy transition state
- Rate of product formation as a function of substrate – initially linear, but eventually becomes constant at high substrate because all of the enzymes have substrate bound – saturated.

Competitive inhibitors:

- Similar in structure to the substrate
- Bind at the active site, compete for substrate binding.
- Substrate can't bind – no product formed.
- Examples are HIV protease inhibitors, statins.

Allosteric Inhibitors:

- Bind to the enzyme at a different site than the active site
- Cause a change in the shape or conformation of the enzyme, so that it is no longer active.
- Can be "feedback inhibitors" in regulation.
- Phosphorylation of the enzyme can cause allosteric change, turning the enzyme on or off.

Covalent Inhibitors:

- Form a covalent bond to the enzyme in the active site, inactivating the enzyme.
- Examples are aspirin, penicillin.

HIV:

- Know the overall life cycle:
  - RNA to dsDNA using reverse transcriptase
  - Integration into host chromosome
  - expression of proteins, followed by maturation of proteins by HIV protease.

HIV Protease:

- Relate the effectiveness of a protease inhibitor to the interaction between the drug and the enzyme, considering: van der Waals, hydrogen bonding, hydrophobic, electrostatic interactions.
- Come prepared to suggest changes to a drug, based on alteration of residues in HIV protease (you will *not* be asked to determine K_i)

Nucleic Acid Structure:

- Distinguish chemical structure of RNA from DNA (ribose/deoxy ribose, U versus T).
- Overall structure of double stranded RNA and DNA: backbone=sugar+phosphate, sidechain=bases, antiparallel strands, phosphates and sugars on the outside, bases on the inside. Sugars connected by phosphodiester bonds.
- Nomenclature of DNA sequences, bases written 5' to 3'. Parity to proteins – amino terminus to carboxy terminus.

Polymerases:

- Mechanism of chain elongation:
  - Primer required, anneals to template via Watson Crick hydrogen bonds
  - dNTPs added to 3'OH of primer, growth of chain is in the 5' to 3' direction
- Error correction, some polymerases have 3' to 5' exonuclease activity to correct errors
- HIV reverse transcriptase lacks this proofreading activity, therefore makes mutations in its own genetic material.
- Mutations cause changes in the drug binding sites of HIV protease and reverse transcriptase, reducing binding of drugs.

Studying Drug resistant HIV Mutations – Overall steps in producing proteins from the mutant gene in E. coli

i. Isolation of viral RNA
ii. Conversion of viral RNA to dsDNA
iii. PCR amplification of desired gene (e.g. protease).
iv. Cutting with restriction enzymes, ligation into plasmid
v. DNA sequencing of mutant gene
vi. Expression and purification of protein.

PCR:

- Reversible conversion of double stranded primer-template to single stranded DNA by heat, re-annealing by cooling, primer anneals to the same location. Each cycle doubles the amount of PCR product. You will ***not*** be asked to give the sequence for primers.
- Uses
  - Addition of restriction sites on end of PCR product to make sticky ends to insert into plasmids.
  - Use in the detection of different number of tandem repeats for the identification of individuals.

DNA Sequencing:

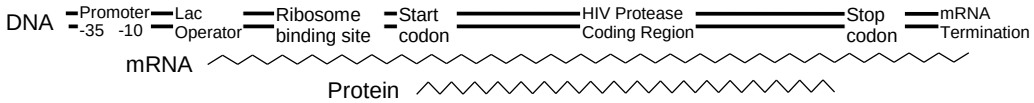
- Use of dideoxyNTPs (ddNTP) to terminate chain at known base.
- Reading of sequencing trace.
- Establishing reading frame from known DNA sequence.
- Using codon table to covert DNA sequence to protein sequence.

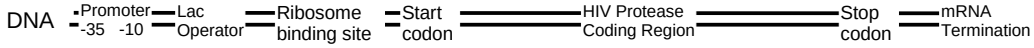
Restriction Endonucleases & DNA ligase:

- Recognize DNA sequences that are the same (5'-3') on the top and bottom strand.
- Cut both strands at the same location.
- Produce sticky ends if the cut site is not in the center.

- You are ***not*** required to memorize any restriction sequences.
- Given the shorthand notation (e.g. GGG^CCC) be able to write the double stranded products of the reaction.
- Use of complementary Watson Crick basepairing of sticky ends and DNA ligase to reform phosphodiester bond.
- Using restriction enzymes and DNA ligase to insert PCR product into plasmid.
  - Sites on the PCR product have to match the sites on the plasmid
  - PCR product is digested with enzymes, plasmid is digested with same enzymes
  - DNAs are mixed, and DNA ligase joins the PCR product to the plasmid.

Summary of Expression Features:





mRNA production (DNA to mRNA)	X	X				X
Protein production (mRNA to protein)			X	X ——codons——→	X	

Role of each for the following DNA segments:

Antibiotic resistance gene: Codes for a protein that can inactivate an antibiotic.

Promoter: Site of RNA polymerase binding

Lac Operator: Binding site for lac repressor protein, prevents generation of mRNA when lac repressor is bound

Ribosome binding site, start codon, stop codon: Required for protein synthesis – see below.

Protein synthesis:

- Overall structure of the ribosome, 30s, 50s subunits, role of each subunit, exit tunnel
- Positioning of Met codon due to spacing from the ribosome binding site.
- Cycle of adding new amino acids
- Role of stop codon in release of protein.
- Inhibition of protein synthesis by antibiotics – modes of action, but not the names of antibiotics.

Carbohydrates:

- 5 carbon sugar –ribose, found in nucleic acid, 6 carbon sugars– glucose
- Storage polysaccharides (starch, glycogen). Shorthand nomenclature for linkage (e.g. β(1-4)).
- Structural polysaccharides: Cellulose, bacterial cell walls.

Metabolism:

- General properties of pathways. Regulation by feedback inhibition and enzyme phosphorylation.
- Energy currency: carbon oxidation, NADH & $FADH_2$ as electron carriers, hydrogen ion gradient, ATP syn.
- Glycolysis:
  - Input- glucose, output – pyruvate,

  - Ultimately produces ATP
  - regulation of phosphofructokinase by ATP/ADP.
  - Location cytoplasm/cytosol.
- TCA cycle:
  - Input – pyruvate (acetyl CoA), output – CO_2,
  - many oxidation steps resulting in NADH & $FADH_2$
  - Cyclic pathway
  - Location: mitochondrial matrix.
- Electron transport:
  - Input – NADH & $FADH_2$.
  - Four complexes.
  - Oxygen is final electron acceptor.
  - Energy released by transfer of electrons to oxygen captured in a hydrogen ion gradient across the inner mitochondrial membrane.
  - Electron transport chain is localized in the inner mitochondrial membrane.
- ATP synthesis.
  - Input – ADP, inorganic phosphate (Pi) and proton gradient.
  - Flow of protons from high concentration to low across the inner mitochondrial membrane, causes conformational changes in ATP synthase that result in the addition of Pi to ADP to form ATP.
- Use of ATP as energy source – some examples:
  - DNA replication – separation of strands by helicase
  - Kinesin – chromosomal separation

Lipids & Membranes:

- Understand the relationships between the hydrophobic effect and spontaneous formation of micelles and bilayers.
- How does a genetic deficiency in the LDL receptor lead to significant medical problems related to excess cholesterol (reduces feedback regulation of cholesterol biosynthesis). What is the general mechanisms by which statins help correct his problem?
- Be able to distinguish between transport proteins and signaling proteins, integral membrane proteins (e.g. G-protein coupled receptor) and membrane anchored proteins (e.g. heavy chain of antibody).
- You should know the function of the following structures in the cell:

ribosome	golgi
mitochondria	centrosome
rough endoplasmic reticulum	nucleus

- Mechanism by which proteins are exported out of the cell. Signal peptide sequence and stop transfer sequence. How would you design a bacterial expression system to export proteins outside of the cell?

Signaling and Antibody Therapy:

- Regulation of glucose metabolism by G-protein coupled receptors (glucagon, epinephrine) and tyrosine kinase coupled receptors (insulin). How phosphorylation and dephosphorylation of glycogen synthase and glycogen phosphorylase affect the incorporation of glucose into glycogen.
- Treatment of Her2 breast cancer with antibodies.
- Overall structure of antibodies (heavy and light chains, variable and constant domains, hypervariable loops) and basis of recognition of the antigen by the antibody. Alternative splicing controls whether antibody is soluble or membrane bound.

Genetics and Gene Regulation

- **Overall structure of chromosomes.**
  - kinetochore
  - centromere
  - chromatids
  - sister chromatids.
- Distinction between autosomal and sex chromosomes. Concept of homologous chromosomes in diploid organisms like humans. XX = female, XY= male.
- Condensation of DNA around histones (forming nucleosomes) & regulation by histone modification.
- Replication of telomeres by telomerase – shifting RNA template provided by telomerase.

mRNA splicing – Alternate splicing generates diversity in *eukaryotic* proteins. Controls whether heavy chain (and attached light chain) will be released as soluble antibodies (plasma cells) or remain attached to membrane (B-cells).

Gene Regulation:

- Activators of transcription (mRNA production)
- Repressors of transcription (mRNA production)
- Histone modification (eukaryotic cells only)
- mRNA stability (polyA tail addition)

Mitosis (2n →2n, DNA is an exact copy).

- Different steps (DNA synthesis, interphase, prophase, metaphase, anaphase, telophase).
- Separation of <u>chromatids</u> by spindle fibers (organized by centrosomes)
- Progression through cell cycle driven by waves of cyclin expression (no need to memorize order of cyclin expression, just how the wave drives the cell cycle.)

Meiosis (2n→n, Recombination generates novel sequences in the gametes)

1. Meiosis I:
   - Alignment of homologous chromosomes, crossing over, or exchange of DNA between homologous chromosomes**, generating new sequences that were not found on the original chromosomes.**
   - Separation of <u>chromosomes</u> by spindle fibers, organized at the centrosome.
2. Meiosis II:
   - Alignment of chromosomes, separation of <u>chromatids</u>.

Anuploidy.

- Non-disjunction of chromosomes causes genetic diseases.
- monosomy, trisomy can occur for both autosomal and sex chromosomes.

Microtubules

- Long polymers generated from subunits.
- Subunits add to ends of filaments. Depending on the concentration of the monomers, the filament can grow at both ends, or only one. Kinesin is a motor protein that uses the energy from the hydrolysis of ATP to walk along microtubules.
- Loss of tubulin subunits from the kinetochore brings chromosomes to the centromere, motor protein keeps chromosome attached to microtubule (chromosome "climbs" up shrinking microtubule)
- Taxol inhibits microtubule assemble, interferes with growth of cancer cells.

Mendelian Genetics:

- Definition of trait, phenotype, allele, dominant, recessive, genotype.
- Difference between a monohybrid and dihybrid cross, how linkage will affect the outcome of dihybrid cross.
- Sex linked – different outcome from a reciprocal cross. Trait is usually on the X-chromosome since there are few genes on the smaller Y.

Human Inheritance: Can you determine the pattern of inheritance from a pedigree?

- Codominance, how this is expressed. Example was blood groups. What happens when the blood being given is incompatible?
- Rh factor, and complications that arise with pregnancy with Rh+ child.
- Autosomal recessive:
    - Carrier (Rr), has normal phenotype, need to be rr to observe phenotype.
    - Phenotype can appear in children even when two parents are normal (Rr X Rr → rr).
    - Males and females equally affected.
- Autosomal dominant:
    - Phenotype is observed in both Rr and RR
    - Children of parents with phenotype will show phenotype.
    - Parents that do not have the phenotype cannot have children with the phenotype.
    - Males and females equally affected.
- X-linked recessive
    - Phenotype usually observed in males, since only one copy of the recessive allele is required to see the phenotype, Females would require two copies of the same allele, most alleles are rare.
    - Female children of carriers can be carriers.
    - Males can never pass on phenotype to male children, the X chromosome in males is always maternal (comes from the mother).