Exam Coverage: Lectures 10- 18 Antibody to Restriction Enzymes/Ligases

Antibodies:

- Detoxification
- Cancer Therapy
- Production of antibodies (role of B-cells, T_H cells, plasma cells)

Vaccines:

- Passive versus active immunization
- Why new flu vaccines are necessary each year (shuffling of RNA chromosomes)
- Concept of herd immunity.
- Criteria for vaccine development (lethal disease whose symptoms develop slowly)

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
- Decrease in the energy of the transition state is due to the fact that reactants are pre-ordered in enzymatic reactions, so no unfavorable decrease in entropy
- 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.
- Effect of temperature and pH on activity.

Competitive inhibitors:

- Similar in structure to the substrate
- Bind at the active site, prevent (compete for substrate binding)
- Therefore, substrate can't bind – no product formed.
- Ineffective at high substrate

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- Cause a change in the shape or conformation of the enzyme, so that it is no longer active.
- Effective at high substrate

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.
- Why reverse transcriptase and HIV protease are good drug targets (unique to viral life cycle)
- HIV reverse transcriptase inhibitors are both allosteric and competitive. Competitive inhibitor also causes chain termination.

HIV Protease:

- Overall structure of the enzyme (homodimer)
- Active site:
    - Twin aspartic acid residues (25, 25') do cleavage of peptide bond
    - valine 82 and other non-polar residues define specificity – what binds well to active site.
- Determine the relative effectiveness of a protease inhibitor from a reciprocal plot (calculate K_I).
- 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.

Nucleic Acid Structure:

- Distinguish chemical structure of RNA from DNA (ribose/deoxy ribose, U versus T).
- Distinguish purine from a pyrimidine
- Recognize hydrogen bond donors and acceptors on bases, both Watson Crick and others.

- 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'.

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, based added according to Watson-Crick hydrogen bonds and purine-pyrimidine matching.
- 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 the binding of drugs.
- Mutations can cause genetic diseases in general, e.g. sickle cell anemia

DNA sequencing:

- Primer used to generate DNA fragments that all start at same location.
- Use of "colored" dideoxy nucleotides to identify the last base added when the chain was terminated.
- Separation of terminated DNA fragments by size gives the position where the colored dd-base was added.
- Come prepared to:
  - Read DNA sequence data of a wild-type (naturally occurring) and mutant protein.
  - Establish the reading frame given the known protein & DNA sequence of the wild-type enzyme
  - Convert the DNA sequence to protein sequence using a codon table.

PCR

- Reversible conversion of double stranded primer-template to single stranded DNA by heat, re-annealing by cooling, primer anneals to the same location.
- Primer design to amplify a region:
  - Left primer is exactly the sequence of the upper strand.
  - Right primer is exactly the sequence of the lower strand.
  - Adding bases (restriction sites) to ends of the PCR product accomplished by placing bases at the 5' end of the primer.
- PCR cycle: denaturation, annealing, polymerization. Each cycle doubles the amount of PCR product.
- PCR can also be used to make any change in a DNA.

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.
- DNA ligase can be used to rejoin fragments cut by restriction enzyme due to the presence of sticky ends.