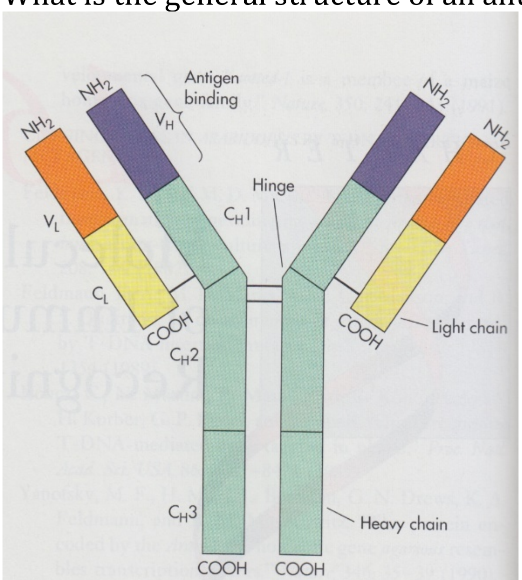
BPS 2110
Assignment 10

1. The human immune system uses a system of "distributed" control.
   a. What are the 5 "design considerations" for the immune system?
      - System must have a way to identify when infection occurs
      - Impossible to "know" beforehand what will infect
      - System must have a way to adapt
      - System must respond before the host animal dies
      - If a microbe infects once, it is likely to infect again
   b. Why does the body use a semi-random method of establishing antibody structure?
      - The immune system does not "know" beforehand what will infect
      - Pathogens are constantly changing
      - There is nobody in charge who can design an antibody, the system has no intelligence
      - If a large enough collection of different molecules are made, random chance dictates that some will have an affinity (bind to) the invading microbe
   c. Why is the system <u>semi</u>-random?
      - The genes for the variable part of immune components are made by combining small gene sequences together.
      - These small sequences are part of the genome of the overall organism
      - The combinations are random, the sequences are not
   d. What is the general structure of an antibody?



   e. How does each B cell ensure that all the antibodies it produces are the same?
      - During growth, each B cell establishes its own "custom" gene to make its antibody
      - Once established within a cell, this gene does not change

- All of the antibodies that are produced by this cell are made using the same "custom" gene

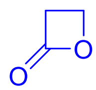
f. How does each B cell produce a different antibody from other B cells?
    - During growth, each B cell establishes its own "custom" gene to make its antibody
    - The process of making this "custom" gene involves random combinations of gene segments.
    - Because the combining process is random, each cell can end up with a different sequence in the "custom" gene

g. How does the immune system "fine tune" antibody structure during an infection?
    - B cells that carry antibodies that are able to recognize invading cells start to replicate
    - During cell division, daughter cells make small changes to the "custom" gene
    - New versions of the B cell that bind better to the invader are selected for further replication

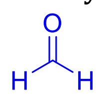
2. Vaccines are used to prevent illness.
    a. What is the general principle of a vaccine?
        - Inject a weakened or dead version of the microbe of interest into the animal or person
        - Weakened microbe will cause a mild infection and trigger immune response
        - Dead microbe will not cause infection but will trigger an immune response (immune system responds to chemicals, not to "life")
        - Immune response increases the population of cells that are specific to the microbe
        - Immune response optimizes the binding between the immune molecules and the invading microbe
        - Normal immune function produces memory cells as the infection is cleared
        - Subsequent exposure to microbe will not cause illness because the memory cells produce an immediate, specific and strong response to the invaders
    b. Why is it necessary to use this method to produce immunity?
        - There is no "design" involved in antibody production
        - Immune system makes random collections of antibodies and relies on "self-selection" to identify best binding antibodies
        - Optimization of antibody structure involves random changes to antibody structure and self-selection
        - Easiest way to manipulate this system is to trick the immune system into producing memory cells
    c. What are the 3 general types of anti-viral vaccine used today?
        - Attenuated virus
        - Killed virus (may or may not be split)
        - Recombinant viral protein

d. Which of the 3 general types listed in part c is (in principle) the safest and why?
   - Recombinant viral protein
   - Does not contain any genetic material, cannot be replicated inside the host to cause an infection
   - Proteins used can be chosen to minimize interactions with the host (avoid exotoxins)

e. What reagents are commonly used to make killed virus vaccines?

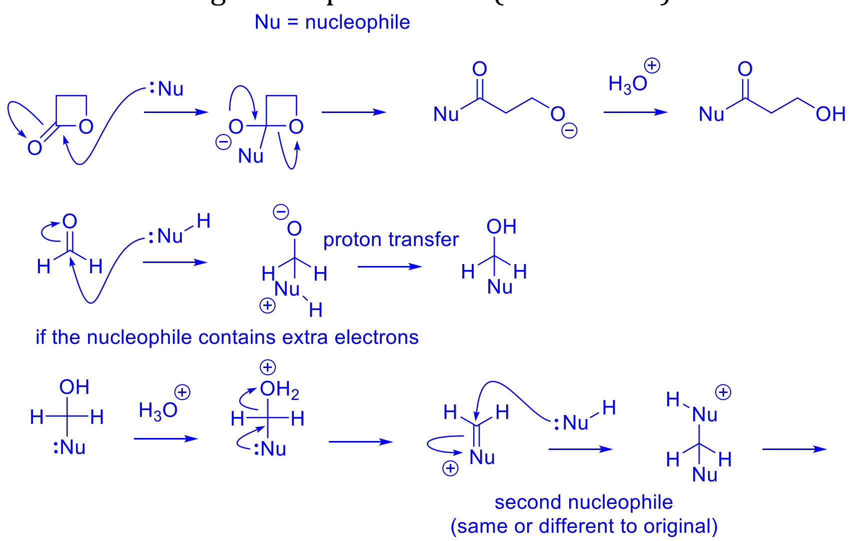


BPL
β-propriolactone)

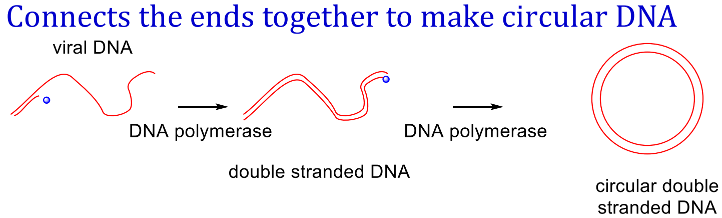


formaldehyde
(formalin)

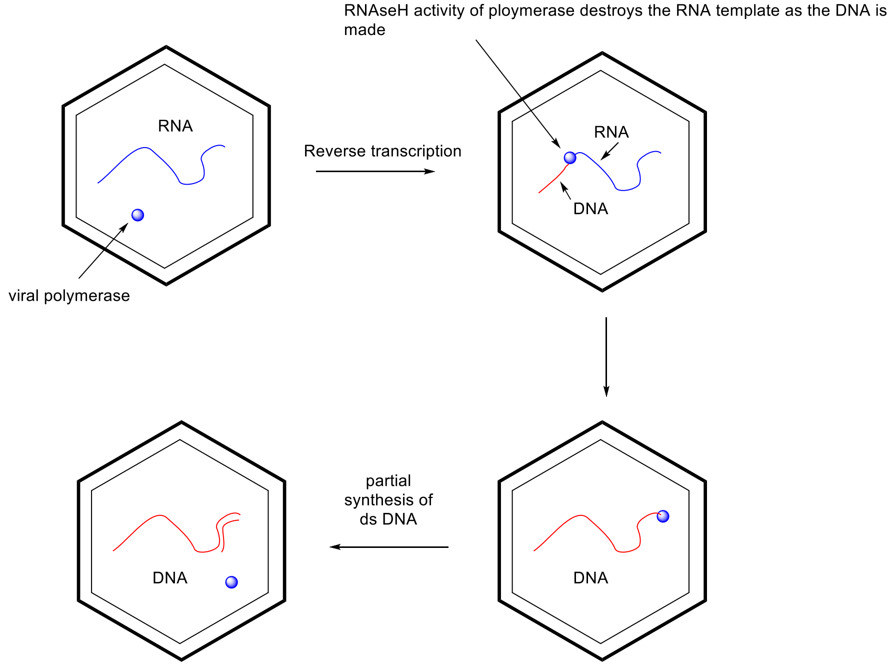
f. How do the reagents in part e work (mechanism)



3. The hepatitis B virus was discovered in 1970
   a. What prior discovery led to the identification of the virus?
      - Discovery of the surface antigen protein (HBsAg) in human blood
   b. What is unusual about the genetic material of the virus?
      - Partly double stranded DNA
      - Mostly single stranded
      - Small section of double stranded
   c. What functions does the viral DNA polymerase carry out during the early stages of infection?
      - Extends the second stranded segment of the viral DNA to make fully double-stranded DNA
      - Connects the ends together to make circular DNA



d. What functions does the viral DNA polymerase carry out during the maturation of the virus?
   - Reverse-transcribes the RNA to make single-stranded DNA
   - Removes RNA nucleotides one at a time (RNAse H) to destroy the RNA template
   - Synthesizes a small section of double-stranded DNA



e. Do you think it would be possible to create a small-molecule drug to combat the virus?
   - Yes. The virus carries at least one enzyme (DNA polymerase). Possible to make enzyme inhibitors

f. If so, what kind of drug could be used?
   - Use a nucleoside analogue
   - Non-natural base
   - Non-natural sugar
   - Best chance of success with non-natural sugar or chain terminator

g. What kind of drug development strategy would be employed to develop this drug?
   - Rational drug design
   - Make a transition state mimic

4. The hepatitis B vaccine was the first anti-viral vaccine to use a protein antigen.
   a. When the hepatitis B vaccine was being developed, why was it impractical to make attenuated or killed versions?
      - Virus only grows inside liver cells
      - Liver cells are very difficult to grow in vitro
      - Difficult to make large quantities of liver cells
   b. What part of the virus is the protein taken from?
      - Outer envelope
   c. Why would a vaccine using this protein potentially be effective?

- Protein is immunogenic (reacts with immune system)
- Protein is on the outside of the virus
- At this location, it is most likely come into contact with immune molecules and cells

d. What was the source of the protein used to make the vaccine?
   - Human blood from persons with chronic hepatitis B infections

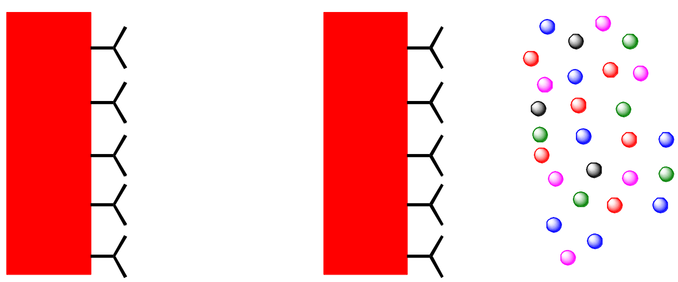
e. Why was it necessary to use this source?
   - Virus could not be grow in large quantities
   - Protein could not be made using chemical methods
   - Recombinant technology was not yet available to make the protein

f. What were the drawbacks to using this method of vaccine manufacture?
   - Hepatitis B is spread by direct exchange of body fluids
   - Other microbes can also be spread this way
   - Persons who became infected with hepatitis B could have also been infected with other pathogens spread via body fluids

5. Describe how affinity chromatography can be used to purify proteins

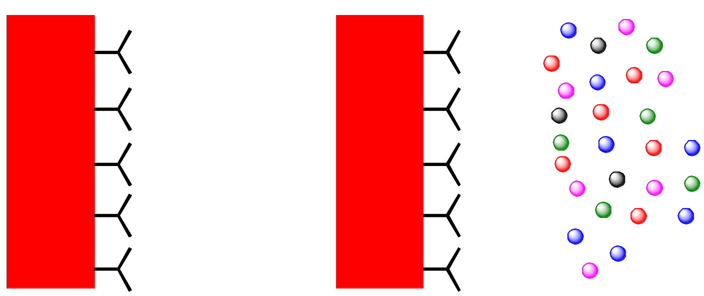
1. attach molecule that has specific affinity for other molecules to solid phase

2. add mixture containing target molecule

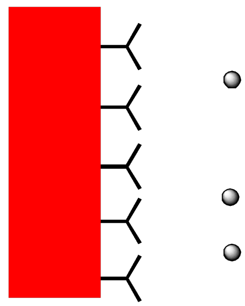


1. attach molecule that has specific affinity for other molecules to solid phase

2. add mixture containing target molecule

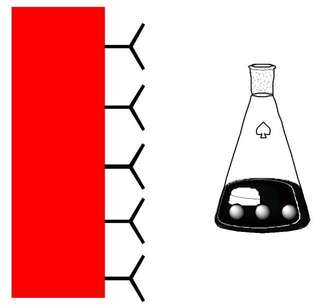


5. add solution to remove bound molecules



change pH, non-polar solvent, change ionic strength

6. separate solid and liquid



6. Plasmids used in genetic engineering often contain genes for antibiotic resistance or amino acid synthesis?
    a. Why are these genes included in the plasmids?
        - After transformation, need a way to separate cells that have taken up the new DNA from cells which have not
        - By growing the cells in a medium that is designed to require the extra genes, the cells which do not take up the DNA die leaving behind only the cells which take up the DNA
    b. Describe how an antibiotic gene helps to achieve the goal in part a?
        - Grow the bacteria in a medium which contains the antibiotic
        - Cells which do not take up the DNA will be killed by the antibiotic
        - Cells which take up the DNA express a gene making them resistant to the antibiotic. These cells live
    c. Describe how a gene for an enzyme making a specific amino acid helps to achieve the goal in part a?
        - Grow the cells in a medium which does not have the amino acid
        - Cells which do not take up the DNA will die because they cannot make proteins without the amino acid
        - Cells which take up the DNA express a gene which provides an enzyme for making the amino acid. These cells are able to live
    d. What amino acid was used during the expression of the hepatitis B vaccine, and how was this used?
        - Leucine
        - Cells were grown using a medium which lacked leucine
        - Cells that did not take up the DNA could not make any proteins containing leucine and died