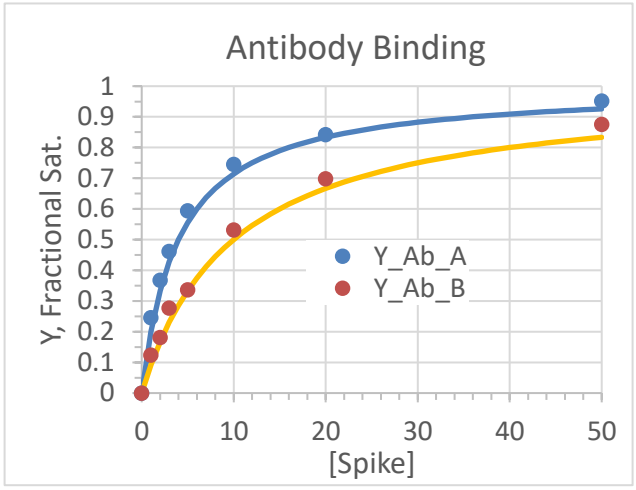
1. Two different antibodies (Ab_A, Ab_B) are being tested as treatments for Covid-19 infection. Both of these antibodies bind to a surface protein on the virus, the spike protein, preventing the virus from entering the cell (physical blocking). A plot of fractional saturation versus the concentration of the spike protein (in nM) is shown on the right. Please answer the following questions.



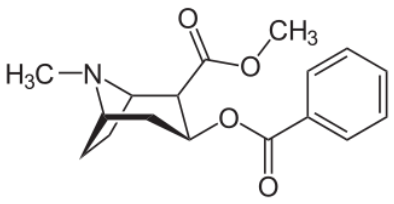
i) What is the K_D for each antibody?

ii) Which of these antibodies will be more effective against Covid-19? You can assume that the concentration of spike protein is 10 nM during an infection. Justify your answer.

i) The Kd is the amount of ligand (spike protein in this case) to ½ saturate the protein. For A, it is about 2 and for B it is about 10.

ii) Antibody A will bind more spike protein at that concentration, 73% versus about 50% for B, so it should be more effective.

2. The Jmol page contains the structure of a complex between an immunoglobulin (antibody) and cocaine. The chemical structure of cocaine is shown on the right. Only the very top part of the immunoglobulin (Fv region) is shown on the Jmol page.



i) Describe the energetics of the interaction between Tryptophan33H and the bound cocaine. Your answer should discuss what stabilizes the bound cocaine, e.g. H-bonds, electrostatics, van der Waals, or the hydrophobic effect.

ii) Describe the interaction(s) between Tyrosine32L and the bound cocaine. Your answer should discuss what stabilizes the bound cocaine, e.g. H-bonds, electrostatics, van der Waals, or the hydrophobic effect.

iii) How would changing tyrosine32L to phenylalanine affect the affinity of cocaine to the antibody? Would the cocaine binding be stronger or weaker? Justify your answer.

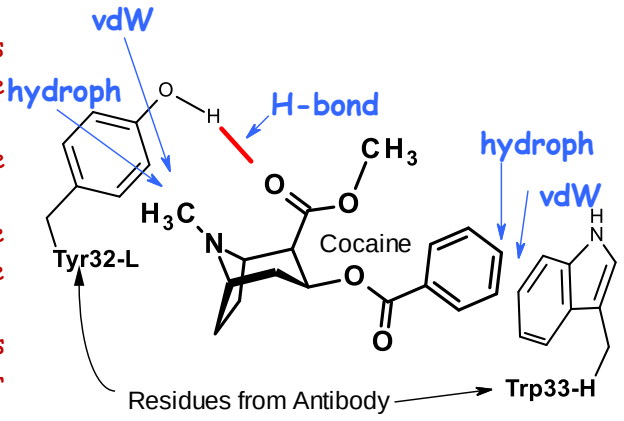
https://www.andrew.cmu.edu/user/rule/03_131/Pset/PS03/ps03_jmol_b.html

You want to consider if any of the following interactions occur between the bound antigen (cocaine) and the antibody:

H-bonds: Are donors and acceptors present in the appropriate location?

Van der Waals: Is there close contact between the antigen and the amino acid side chains from the antibody.

Hydrophobic effect: Are there non-polar surfaces that would lead to the release of ordered water when the antigen binds?



Electrostatics: Are there complementary (opposite) charges on the antigen and the antibody?

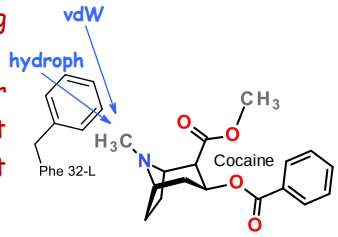
In general, there will always be van der Waals due to shape complementarity, and then one or more of the other three.

i) Tryptophan 33 on the heavy chain is in close contact with the phenyl ring on cocaine, showing van der Waals and the hydrophobic effect.

ii) Tyr32L on the light chain forms an H-bond with cocaine, it also has van der Waals interactions and a hydrophobic interaction with the methyl group on cocaine.

iii) If Tyr32 was replaced by Phe, the -O-H group would be removed, leading to a loss of a hydrogen bond, reducing the affinity.

In summary: The bound cocaine is stabilized by hydrogen bonding, van der Waals and the hydrophobic effect. Cocaine has no ionizable groups, so it will not be charged and therefore electrostatic interactions are not important here.



3. What disease is the drug Trastuzumab used to treat? Briefly describe how it works to cure the patient (*please use the web and provide the appropriate citation*).

Trastuzumab is an antibody that is used to treat breast and stomach cancer. It binds to the HER2 receptor which is a growth factor receptor. This receptor is over-expressed in these cancers, leading to increased growth of the cancer cell in response to normal levels of the growth hormone. The antibody prevents the growth hormone from binding to the receptor, therefore preventing growth of the cancer cell.

Source: Wikipedia

4. Write a short paragraph on Hers' disease. Your essay should discuss:

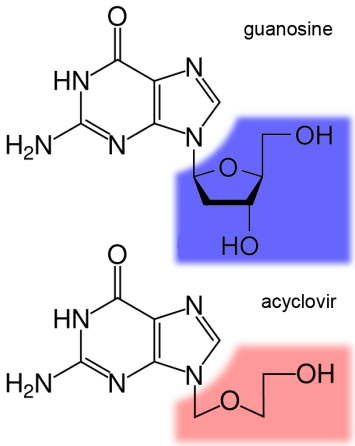
i) The normal function of the enzyme that is affected by this genetic disease.

ii) The consequence of loss of function to the individual.

(*please use the web and provide the appropriate citation*).

This is a glycogen storage disease where the individual is missing glycogen phosphorylase, the enzyme that releases glucose from glycogen. It is normally regulated by the hormone glucagon, which phosphorylates the enzyme.

Surprisingly, this deficiency is relative benign, with an enlarged liver and some growth retardation.



5. Acyclovir is an antiviral agent. The structure of acyclovir and guanosine (deoxy) are shown on the right.

i) Use the web to find out what kind of viral infections are typically treated with acyclovir.

ii) How do you think acyclovir works to inhibit replication of the virus?

i) Herpes simplex.

ii) It is missing the 3' -OH so it cannot form phosphodiester bonds after it is added to the growing chain (addition to the chain requires the phosphorylation of acyclovir to make the triphosphate.

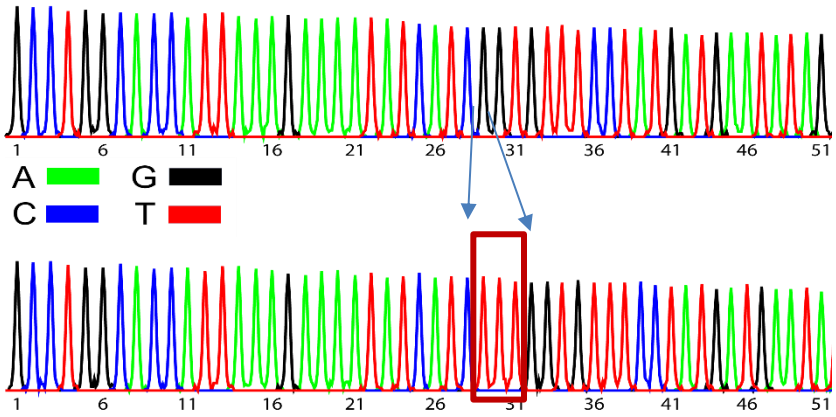
6. Cystic fibrosis is a genetic disease that is caused by a single mutation in a chloride ion transporter (CFTR). The transporter is necessary to move Cl ions across mucus membranes. The chloride then causes water to move across the membrane to reduce the viscosity of mucus. When the transporter is defective the mucus is very thick, causing difficulty in breathing.

The gene for the CFTR protein is very long (over 180,000 bases). A segment of the DNA that codes for the middle section of the protein, with the codon for Phe507 highlighted in grey, is shown below. The DNA sequencing primer is highlighted in cyan. The translation of this sequence gives the following protein sequence for this region of the protein (these are one letter codes for the amino acids, e.g. F=Phe)

```
tgg att atg cct ggc acc att aaa gaa aat atc atc ttt ggt gtt tcc tat gat gaa tat
 W   I   M   P   G   T   I   K   E   N   I   I   F   G   V   S   Y   D   E   Y
```

The disease is recessive, meaning that an affected individual must have two copies of the mutation to have the disease. Heterozygous individuals do not have the disease but are carriers. There is a one-in-four chance that a child produced from two carriers will have the disease. Therefore, genetic testing for heterozygotes is very important if there is a family history of the disease.

You sequence DNA samples from a heterozygous individual, each sequence corresponds to the gene on one of their chromosomes. One of these sequences corresponds to the normal gene sequence and the other corresponds to the mutation that is responsible for cystic fibrosis. The DNA sequencing data is given to the right.



i) Briefly describe how the 2nd blue peak was generated, give its DNA sequence.

ii) What is the change in DNA sequence (mutation) associated with this disease?

iii) How might this change affect the structure of the protein?

Note, when converting the above DNA sequences to codons, skip the first base, i.e. the first codon is CCT, coding for proline (P). Your translation of the DNA sequence should produce the correct protein sequence.

i) This DNA fragment was generated by the addition of a normal dGTP to the primer, followed by the addition of ddCTP. The ddCTP caused the chain to terminate and it also colored the fragment based on its fluorescence.

ii) The lower trace is the normal DNA sequence, the three T bases that code for Phe507 are present (indicated with the red square.)

iii) The upper trace shows that those three T bases are missing (see arrows), causing a deletion of the Phenylalanine residue. This missing residue affects the folding of the ion channel, making it less functional by preventing it movement of the protein to the cell membrane.

7. Fragile X-syndrome is due to an excessive number of CGG repeats in a gene called FMR1. Normal individuals have between 5 and 40 repeats while affected individuals have more than 200. The beginning of the gene is shown below with the CGG repeats highlighted in yellow and the start codon for the protein in green.

```
  1 ctcagtcagg cgctcagctc cgtttcggtt tcacttccgg tggagggccg cctctgagcg
 61 ggcggcgggc cgacggcgag cgcgggcggc ggcggtgacg gaggcgccgc tgccaggggg
121 cgtgcggcag cgcggcggcg gcggcggcgg cggcggcggc ggaggcggcg gcggcggcgg
181 cggcggcggc ggctgggcct cgagcgcccg cagcccacct ctcgggggcg ggctcccggc
241 gctagcaggg ctgaagagaa gatggaggag ctggtggtgg aagtgcgggg ctccaatggc
```

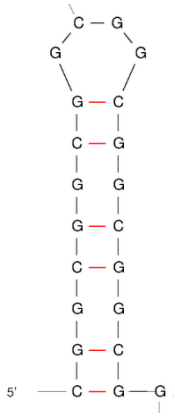
During the normal replication of DNA you would find the following replication structure (the CGG repeats are highlighted and alternate bold/not bold).

```
5′----TGCCAGGGGGCGTGCGGCAGCGCGGCGGCGGCGGCGG
3′----ACGGTCCCCCGCACGCCGTCGCGCCGCCGCCGCCGCCGCCGCCGCCG---5′
```

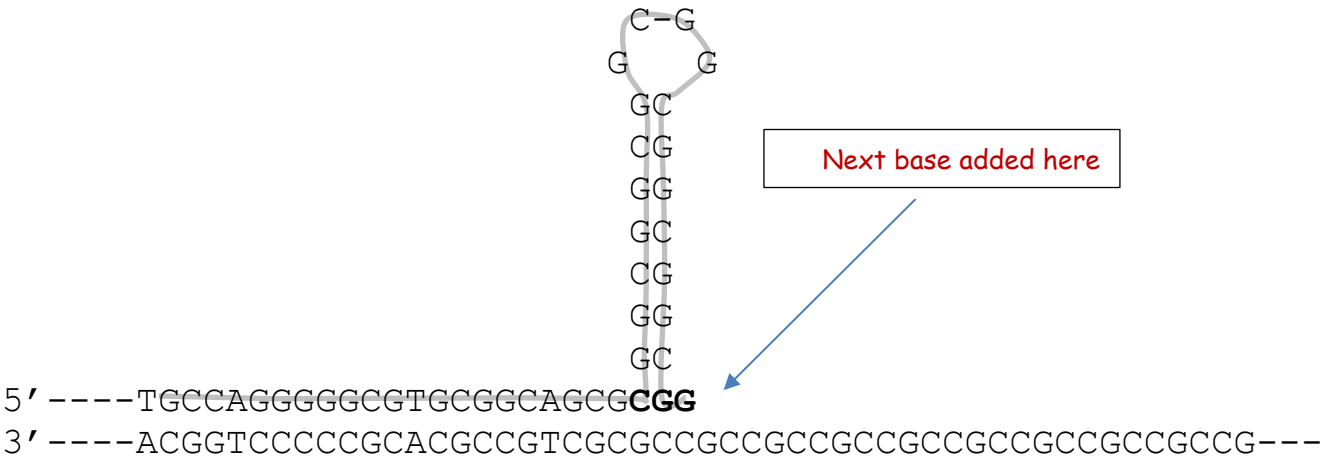
Please answer the following questions:

i) What are the disease symptoms due to fragile x-syndrome (please use the web).
Intellectual disability

ii) Postulate how DNA polymerase activity could increase the number of repeats. As a hint, the CGG repeats can form stabile hairpin structures, as shown on the right. Please ***do not*** use the web to answer this question.



During replication, the strand that is being used as the primer (top strand) can form a hairpin, looping out the DNA. This places the 3′ end at an earlier repeat, after replication the number of repeats has increased. The highlighted region had four repeats.



The looped out material will add more repeats.