1 0.9

0.8

0.2 \prec

0.1

0

0

10

20

Antibody Binding

Y\_Ab\_A

Y Ab B

30

[Spike]

40

50

Trp33-H

- **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<sub>D</sub> 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.



- 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 thehydroph 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.



Residues from Antibody

vdW

- 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.** i) Complete the following table for two of the mutants:

	Residue Altered	Changed to	Affects Specificity (Y/N)	Affects Catalytic efficiency (Y/N)
Trp1	Asp189	Ala	Yes	No
Trp6	Ser195	Gly	No	Yes

ii) For the mutation that affects specificity, suggest how the specificity of the mutant enzyme would be different from the wild-type. Recall that the wild-type likes to bind substrates with lysine sidechains.

The mutant contains a non-polar Ala residue, so it would be more likely to bind peptides with non-polar sidechains, e.g. Phe, Ile, etc.

- **4.** 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. **Note** that Wikipedia is always a good starting point for literature search, **most** of the pages are correct. However, you should also check the sources that are listed on the Wiki page and additional sources to verify critical information that would be important for your studies.
- 5. 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.