1. (10 pts, 15 min) The regulation of the oxygen affinity of Hb by bisphosphoglycerate (BPG) is important in adaptation of oxygen delivery at high altitudes.
   i) Open the Jmol page for this question. The structure of deoxy hemoglobin with BPG bound is shown on the left. The structure of oxy-hemoglobin is shown on the right. In the deoxy structure, measure the distance between the two NE2 atoms on the histidine residues that contact the BPG (shown in spacefill). You can highlight the two atoms on each histidine by checking the "His" box. What is this distance? (2 pts) 1.425 nm or 14.25 A
   ii) Now measure the size of the potential BPG binding pocket on oxy-hemoglobin by measuring the distance between the two NZ atoms on the lysine residues. You can highlight the two atoms on each lysine by checking the "Lys" box. 1.073 or 10.73 A
   iii) Discuss why BPG cannot bind to oxygenated hemoglobin (2 pts).

   The pocket in oxyHb (R state) is too small to fit BPG, so BPG can only bind to the T state.

   iv) If His145 on the β-chains of hemoglobin were replaced by aspartic acid would it be necessary to have much higher, or much lower, BPG level to achieve the same effect on oxygen delivery. Why? [His 145 is one of the His residues from the β-chain that is shown in the diagram of the BPG - hemoglobin complex in lecture 13, it is also the histidine whose atoms are highlighted on the left Jmol page when you click "His"]. (4 pts)

   The BPG binds between the two subunits of Hb by interaction with the positively charged residues on the Hb. This interaction is a favorable electrostatic interaction. If one of the His residues on Hb is replaced by Asp, this would weaken the interaction because Asp has a negative charge. Therefore, a higher concentration of BPG would be required to reach the same amount of bound BPG.

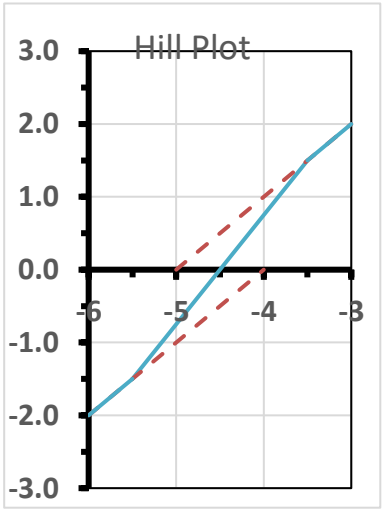
2. (5 pts, 15 min) You are investigating cooperative binding to a protein that has four binding sites for a ligand and you experimentally measure the first (K_{A1}) and last (K_{A4}) values by measuring the binding at very low and very high ligand concentration. These are macroscopic K_A values. You obtain values of 4×10^4 M^{-1} and 0.25×10^4 M^{-1}, i.e. the last K_A is smaller than the first, suggesting weaker binding and negative cooperativity. Is this actually the case?

   The relationship between the macroscopic and microscopic binding events for a tetramer are:

   $K_{A1}=4K_{A1}^{\mu}$ – there are four ways the ligand can bind, so the apparent on-rate is $4 \times k_{on}$. There is only one way for the ligand to come off of each species, so the off-rate is k_{off}.

   $K_{A4}=(1/4)K_{A4}^{\mu}$ – there is only one way the ligand can bind, so the apparent on-rate is k_{on}. Any one of the four ligands can come off, so the apparent off-rate is $4 \times k_{off}$.

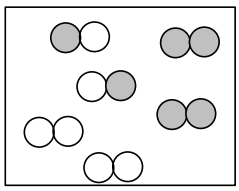
   The microscopic binding constants for the first and last step are the same, 10^4 M^{-1}, the binding is non-cooperative.



3. (6 pts, 15 min) A dimeric protein shows two K_D values, the first K_D is 10^{-4} M and its second K_D is 10^{-5} M. Its Hill coefficient is 1.5.
   i) Sketch, in the diagram on the right, the Hill plot for this protein (2 pts).

   At very low and high ligand concentration the binding will be non-cooperative with a slope of 1 on the Hill plot, the line at low ligand will intersect the x-axis to give the first K_D ($\log K_{D1}$=-4) and the line at high ligand will intersect the x-axis to give the second (last in this case) K_D ($\log K_{D2}$=-5). The central part of the Hill plot will be a line with a slope of 1.5 whose crossing point is at the mid-point between the two $\log K_D$ values. These three line segments will intersect and the complete hill plot is shown in blue.

   ii) Estimate the K_D^{OBS} from your Hill plot, this is the ligand concentration to give Y=0.5 (2 pts). The intersection is at -4.5, so $K_D^{OBS} = 10^{-4.5} = 3.16 \times 10^{-5}$ M.

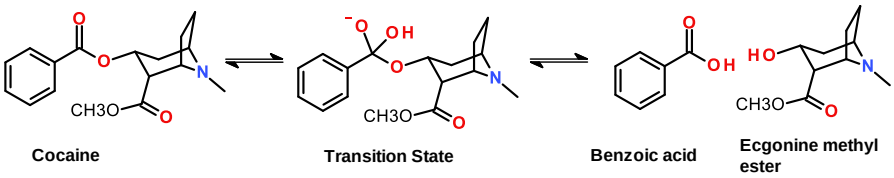


   iii) Indicate the distribution of bound ligands you would expect to see when Y=0.5, using the lower diagram. Briefly justify your drawing (2 pts).

   Since Y=0.5, ½ of the binding sites are occupied. The cooperativity is positive, so there will be more ML_2. It is not infinitely positive, so there will be some ML.

4. (5 pts, 15min) Most enzymes contain functional groups in their active site that assist in performing a chemical reaction. Antibodies also act as catalysts if they preferentially bind to the transition state of the reaction, i.e. the K_D for binding to the transition state is smaller than the K_D for binding to the substrate. These catalytic antibodies are called Abzymes. Abzymes usually do not contain any groups that directly assist in the mechanism, yet they still catalyze the reaction. Explain why these antibodies are catalysts. *[Hint: The reaction rate depends on the concentration of the transition state, how will adding an Abzyme to a solution of substrate affect the concentration of the transition state?]*

Fun fact: Catalytic antibodies can be used to treat cocaine overdose by catalyzing the hydrolysis of cocaine, as shown to the right.



If the antibody binds the transition state it will perturb the equilibrium between (S) and its transition state, generating more transition state. Since the rate of the reaction is proportional to the concentration the transition state the rate of the reaction will increase, both the free transition state and the antibody-transition state complex will contribute to the rate of cocaine degradation.

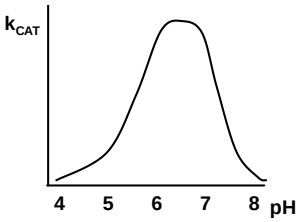
Without antibody: $S \rightarrow X^{\dagger}$

With cat. antibody: $S \rightarrow X^{\dagger} + Ab \rightarrow (Ab\text{-}X^{\dagger})$.

The equilibrium concentrations of free (S) and the free transition state will be the same in both cases, however the overall concentration of the transition state is increased by the additional transition state bound to the antibody. The overall rate is proportional to the sum of both transition states: rate $\approx X^{\dagger} + (Ab\text{-}X^{\dagger})$.

This is another way a protein can increase the rate of the reaction without having any catalytic groups whatsoever.

5. (5 pts, 20 min) A sequence alignment of a number of ribonucleases is shown below. Ribonucleases degrade RNA molecules. The pH dependence of k_{CAT} is shown on the right. You can view the enzyme using JMol using the link on the problem set page. The JMol page provides instructions on how to visualize the location of individual residues, or multiple residues.



Use this information to determine the key catalytic residues for ribonuclease. *Briefly justify your answer*.

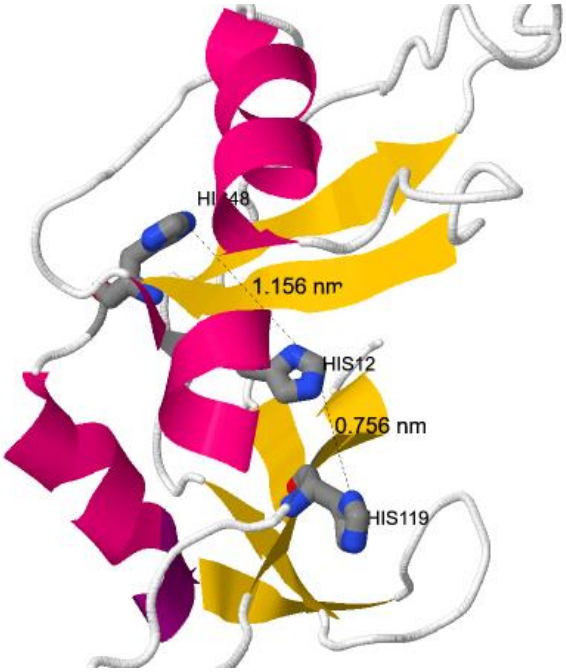
```
                1         10        20        30        40        50
Cow             KETAAAKFERQHMDSS.TSAASSSNYCNQMMKSRNL.TKDRCKPVNTFVHESLADVQAVC
human           KESRAKKFQRQHMDSD.SSPSSSSTYCNQMMRRRNM.TQGRCKPVNTFVHEPLVDVQNVC
Pronghorn       KETAAAKFERQHIDSN.PSSVSSSNYCNQMMKSRNL.TQGRCKPVNTFVHESLADVQAVC
Horse           KESPAMKFERQHMDSG.STSSSNPTYCNQMMKRRNM.TQGWCKPVNTFVHEPLADVQAIC
Bat             KESWAMMFQRQHMDPD.G.PSSNSNYCNQMMRRQLM.TERQCKPVNTFIHEPLVDVQAIC
DOG             RESKAMKFQRQHMDSH.P.AAISASYCNLMMKRRNM.TDGWCKPVNTFVHEPLADVQAVC
Chicken         .VPTYQDFLRTHVDFPKTSFPNIAAYCNVMMVRRGINVHGRCKSLNTFVHTDPRNLNTLC

                60        70        80        90        100       110
Cow             SQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPNCAYKTTQANKHIIVACEGNPYVPV
human           FQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEGSPYVPV
Pronghorn       SQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPNCAYKTTQAKKHIIVACEGNPYVPV
Horse           LQKNITCKNGQSNCYQSSSSMHITDCRLTSGSKYPNCAYQTSQKERHIIVACEGNPYVPV
Bat             LQGNIICKNGKPNCHKSSSSMKITDCRVKSSSEYPFCDYETSHKERHIIVVCGGNPYVPV
DOG             SQKDVLCKNGQSNCHQSRSQMNITDCRLKNGSKFPKCVYTTTQKEQYIVVACEGNPHVPV
Chicken         INQPNR.....ALRTTQQQLPVTDCKLIR.SHPTCSYTGNQFNHRVRVGCWGG..LPV

                120
Cow             HFDASV....
human           HFDASVEDST
Pronghorn       HYDAS.....
Horse           HFDASVEVST
Bat             HFHASVEVST
DOG             HFDACL....
Chicken         HLDGTFP...
```

The pH dependence indicates two ionizable groups with pKa values of 5.5 and 7, suggesting His residues. It might also be possible to have an Asp or Glu with a pKa of 5.5, but this is less likely.

Polar residues that are conserved in all species are:

Arg 10	Asn27	Cys58	Tyr97
His 12	Lys41	Thr82	Cys110
Asp 14	Asn44	Asp83	
Tyr25	Thr45	Cys84	
Cys26	**His48**	Cys95	
His119			

This suggests that His12, His48, or His119 are the catalytic residues. The sidechains of **His12 and His119** are close to each other, as indicated in the JMol, (0.75 nm) and therefore are likely both part of the active site. The sidechain of the third His, His48, is behind secondary structure and more than 1.1 nm from His12, so it is less likely to be involved with catalysis.



Although Asp14 is also conserved and in this region, the pH dependence does not support its role in catalysis.

Note that the Cys residues are involved in disulfide bonds in this protein.

6. (7 points, 10 min) The following question will require you to visualize some trypsin structures using Jmol. The URL to the Jmol page can be found on the problem set page. These enzymes have various mutations (amino acid changes). Depending on your last name, select the appropriate mutant enzyme (i.e. Trp2-Trp6) and answer the following questions.

   Trp2: Moza Al Shurkri, Aya Al-Ansari, Mohammed Al-Lakhen

   Trp3: Asma Al-Maraghi, Amna Al-Sayegh, Lulwa Alhaddad, Alreem Alkhanji

   Trp4: Sara Alyafei, Maryam Aslam, Laila Assami

   Trp5: Mahnoor Fatima, Haher Habboub, Mohammad Hammad

   Trp6: Syeda Hira Hashim, Thamanna Muhammed Hashir, Ayah Salameh, Mohammed Sayed

   i) State which residue was altered in your assigned protein and how it was altered (Sample answer: Asp189 to Ala) (2 pts).

	Residue Altered
Trp1	Asp189 to Ala
Trp2	Asp189 to Glu
Trp3	Asp189 to Lys
Trp4	Asp189 to Met
Trp5	Asp102 to Asn
Trp6	Ser195 to Gly

   ii) *Sketch* both the wild-type (no mutations) and your enzyme, indicating the location of the amino acid change in your particular enzyme. The level of detail that you should provide is indicated in the sketch on the right. Note that this sketch has several **errors and missing functional groups** (e.g. atoms are missing from the sidechain of the substrate). Your sketch should portray the **correct** structure of the enzymes as you would find in the **starting (ES) complex** (5 pts). The diagram for the first mutant (Asp189 to Ala) is shown.