Biochemistry 461, Section I **Your Printed Name:**_____

April 23, 1998

Exam #3 **Your SS#:**_____

Prof. Jason D. Kahn

(typos corrected) **Your Signature:**_____

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Some information which may be useful is provided on the bottom half of this page.

Explanations should be concise and answer the specific question asked.

You probably will not need a calculator for this exam. No other study aids or materials are permitted.

All points are not created equal: the end of this exam is significantly easier than the beginning. Act accordingly.

Possibly Useful Information:

Michaelis-Menten equation: $v_0 = V_{max}[S]/(K_M + [S])$

Type of inhibition	Apparent K_M	Apparent V_{max}	Apparent V_{max}/K_M
Competitive	□K_M	V_{max}	(1/□) V_{max}/K_M
Uncompetitive	(1/□□)K_M	(1/□□)V_{max}	V_{max}/K_M
Mixed	(□/□□)K_M	(1/□□)V_{max}	(1/□) V_{max}/K_M
Noncompetitive (□=□□)	K_M	(1/□) V_{max}	(1/□) V_{max}/K_M

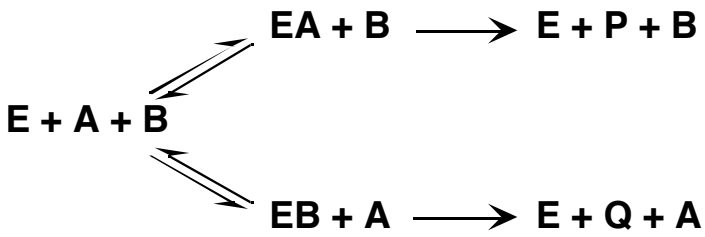
□ = 1 + ([I]/K_I) □□= 1 + ([I]/K_I)□

$V_{max} = k_{cat}$ $[E]_{total}$

$Y_{O_2} = pO_2^n/(p50^n + pO_2^n)$

1. (25 pts) Michaelis-Menten Kinetics.

We have asserted but not demonstrated that the specificity of enzyme action, i.e. the choice of which substrate to use, is described by k_{cat}/K_M. We will derive this relationship using equations for enzyme inhibition. Refer to the kinetic scheme below. The enzyme E, which has one active site, can either convert substrate A to product P (with Michaelis constants $k_{cat,A}$ and $K_{M,A}$) or convert substrate B to product Q ($k_{cat,B}$ and $K_{M,B}$).



(a; 2 pts) In the absence of substrate B, what is the equation relating $v_{0,P}$ for the formation of P to the concentration of A (this is easy).

(b; 4 pts) When both substrates are present, they can be considered to act as inhibitors of each other. Explain why it is reasonable to treat B as a competitive (rather than some other kind of) inhibitor with respect to A and *vice versa*.

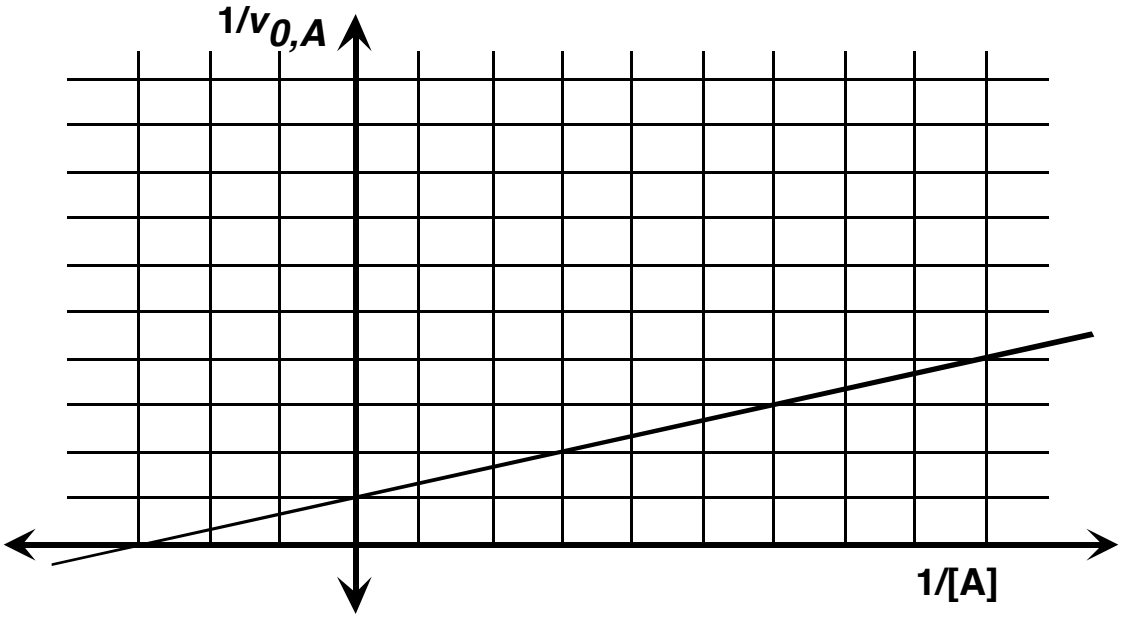
(c; 4 pts) If we're going to treat inhibition, we need to include an α parameter. Instead of using a K_I or K_S as the relevant constant in our equation for α, we will use a K_M, which is not an equilibrium constant. Solve the equation below (which describes the steady-state approximation) for [ES]/([E][S]), and use this result and the definition of K_M to rationalize the choice of K_M mathematically.

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] = 0$$

(d; 6 pts) Using $\alpha = 1 + [\mathrm{B}]/K_{M,B}$ in the equation for competitive inhibition, write down the equation for $v_{0,P}$ for the conversion of A to P in the presence of competing substrate B. Multiply top and bottom by $1/K_{M,A}$. Write down the corresponding equation for $v_{0,Q}$ for the conversion of B to Q. You do not need to have solved b and c above to proceed here.

(e; 5 pts) Finally, divide the equations above to give the desired expression for $v_{0,P}/v_{0,Q}$. This is valid for any concentrations of A, B, and E. Why do we call k_{cat}/K_M the specificity constant?

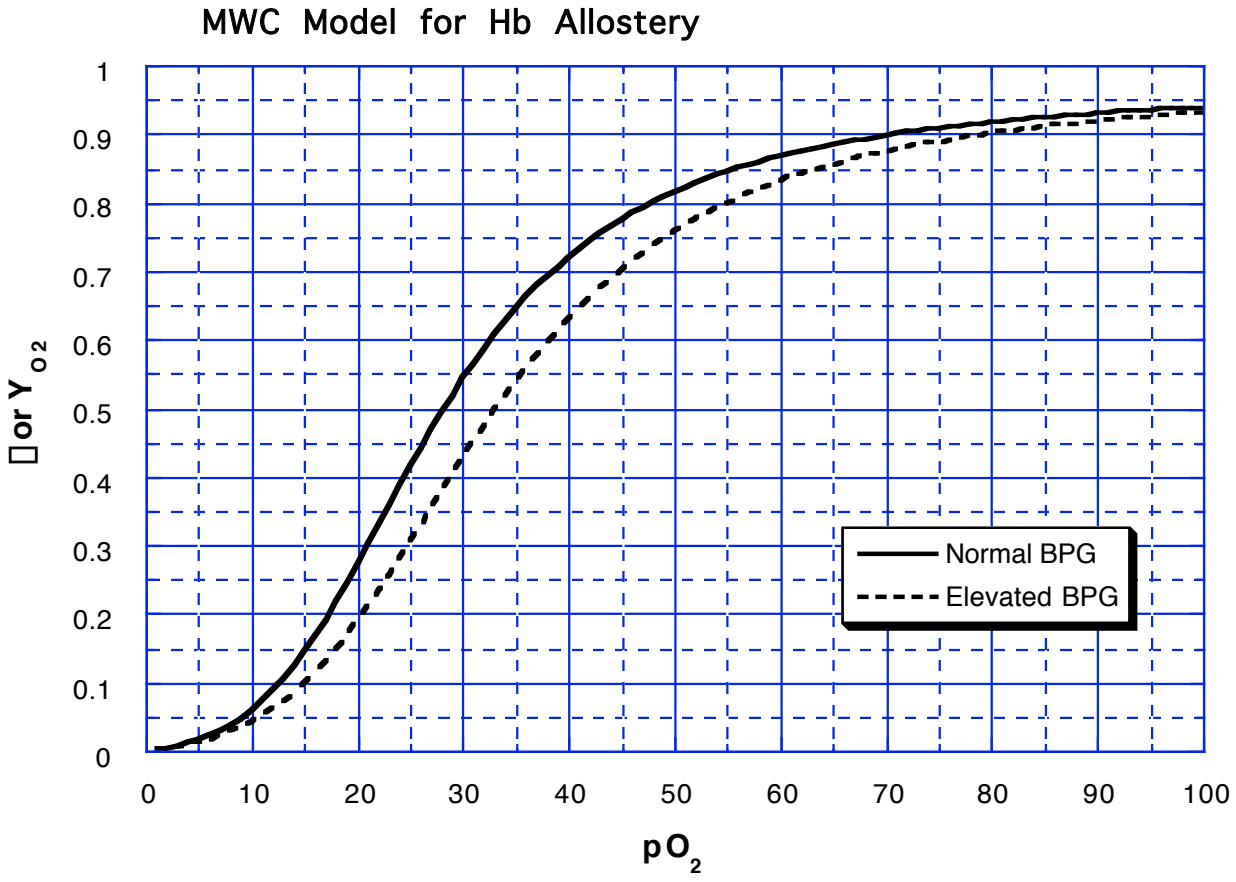
(f; 4 pts) The double-reciprocal plot below relates $1/v_{0,P}$ to the concentration of A in the absence of competing substrate. Draw the line we would get in the presence of B, at [B] = $2K_{M,B}$.



2. (25 points) Hemoglobin Allostery.

2,3-bisphosphoglycerate (BPG) levels in the erythrocyte increase as a response to high altitude. The graph below shows partial saturation with O_2 as a function of pO_2 as usual. At sea level, pO_2 in the air (and thus the lungs) is about 90 Torr. At 4500 m (14000 ft) it is about 55 Torr. The pO_2 in the tissues is about 30 Torr.

(a; 8 pts) <u>How much oxygen (as a percentage of Hb sites, as in class) is delivered to the tissues</u> (1) at sea level in the presence of normal BPG, (2) at 4500 m at normal BPG, and (3) at 4500 m at elevated BPG. Indicate on the graph how you obtained the numbers used in your calculation.



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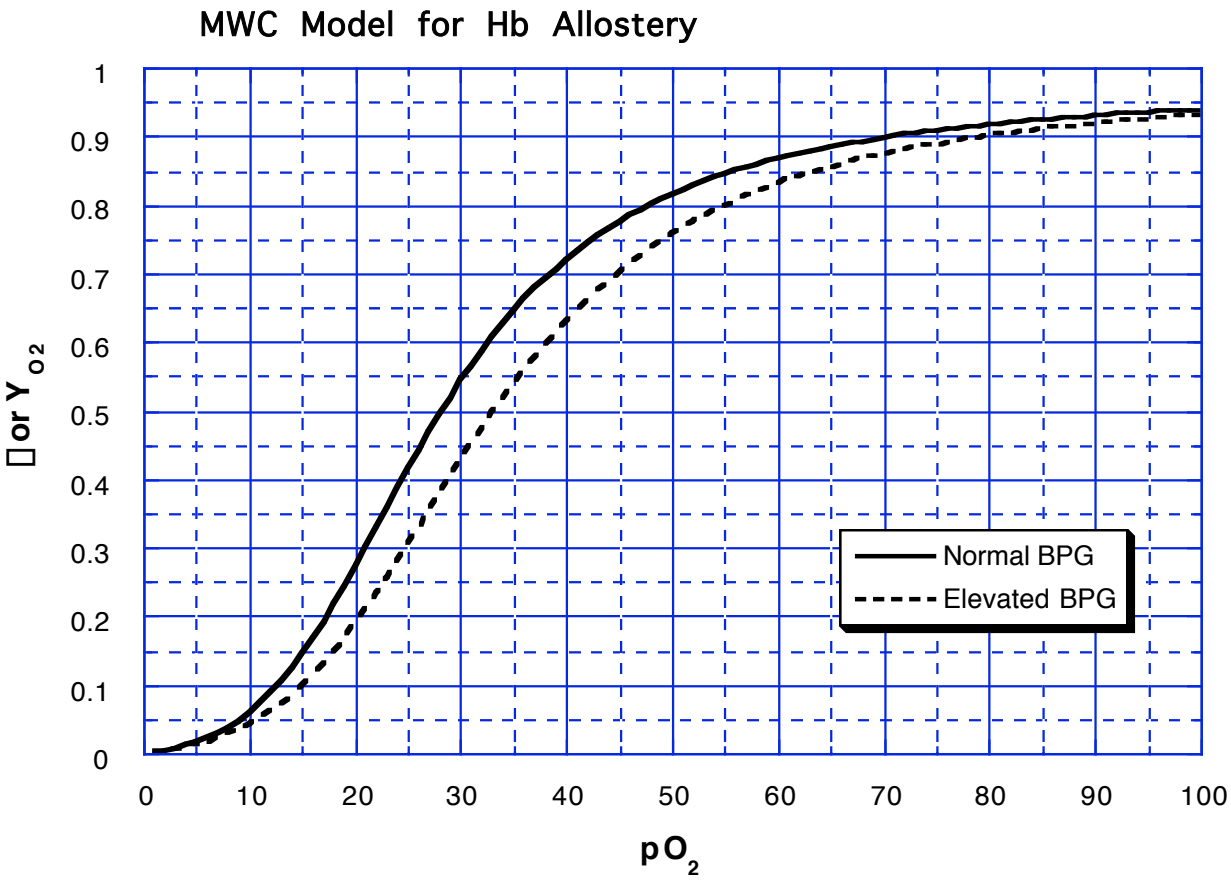


(b; 3 pts) From the above graph, what is p50 in the presence and absence of elevated BPG?

(c; 7 pts) The Bohr effect is the name given to the fact that as pH decreases, oxygen affinity also decreases. On the graph above, draw the curve you would expect for oxygen affinity at elevated BPG and at a pH where p50 is further increased by 7 Torr. What is the physiological rationale for the evolutionary value of the Bohr effect?

(d; 7 pts) Describe the mechanism either of the Bohr effect or the BPG effect (not both) in terms of the quaternary structure differences between the T and R states.

3. (20 points) Methods.

DNA is a polyanion. DNA binding domains tend to interact electrostatically with the phosphate backbone of DNA.

(a; 3 pts) What amino acid residues are likely to be common in DNA binding domains?

(b; 2 pts) Circle a reasonable pI for a DNA binding protein: 3 6.5 9.5

(c; 5 pts) What kind of ion exchange chromatography might you use to purify DNA? What would you use for a DNA binding protein? Briefly explain your reasoning.

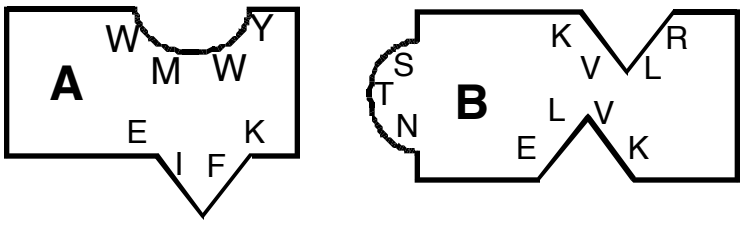
(d; 6 pts) You have isolated a cDNA (a gene) for YFP (your favorite protein), but you don't have purified protein. Briefly describe how you could use molecular biology to purify the protein using an affinity method.

(e; 4 pts) Give two reasons we use SDS in SDS-PAGE to determine the molecular weight of proteins.

4. (15 points) Quaternary Structure.

(a; 3 pts) We have discussed rotational symmetry in quaternary structures. Why do we never observe mirror planes in biological quaternary structures?

(b; 8 pts) For proteins A and B below, draw the most likely A:B dimer and briefly explain sources of specificity in quaternary structure.



(c; 4 pts) Briefly describe any method of determining the stoichiometry of polypeptides in a multisubunit protein.

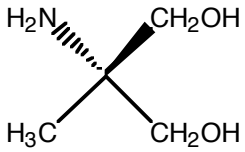
5. (15 points) Fundamentals of Catalysis.

(a; 4 pts) Define a catalyst. Does a catalyst affect thermodynamics, kinetics, or both?

(b; 3 pts) How do the interactions among subunits or between enzyme and substrate compare to the interactions which stabilize protein folding?

(c; 4 pts) What are two restrictions on enzyme catalysts that are not, for example, faced by chemists at Exxon or Dupont?

(d; 4 pts) Draw a picture to explain how an enzyme could recognize just one of the two alcohol groups on this achiral molecule:



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Score:

Question 1: _____ out of 25

Question 2: _____ out of 25

Question 3: _____ out of 20

Question 4: _____ out of 15

Question 5: _____ out of 15

Total: _____ **out of 100**