

Professor Charles McKenna
Professor Peter Qin
Chemistry 519, Spring 2005
Wednesday, March 7, 2005
10:00 a.m. – 11:30 a.m.

NAME: _____

Midterm Exam

GOOD LUCK!

PROBLEM	POSSIBLE POINTS	SCORE
1	30	
2	30	
3	30	
4	30	
5	30	
TOTAL	150	
Bonus	5	

1. (30 pts).

Give one example of an amino acid that has the following properties. For each of the example, show the full name, one letter code, and the chemical structure of the side chain.

- (a) An amino acid whose side chain is positively charged at pH 7.0.
- (b) An amino acid whose charge can vary between pH 5.0 to 8.0.
- (c) An amino acid that has strong fluorescent emission.
- (d) An amino acid that favors *trans* or *cis* configurations equally in the peptide bond.
- (e) An amino acid with a hydrophobic side chain.

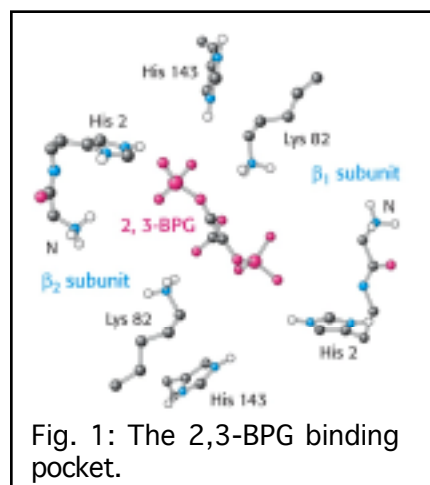
2. (30 pts).

In hemoglobin, the fraction of sites bound with O₂ (Y) depends on the partial O₂ pressure (pO₂) according to:

$$Y = \frac{(pO_2)^n}{K^n + (pO_2)^n} = \frac{(pO_2/K)^n}{1 + (pO_2/K)^n}$$

where K is the dissociation constant between hemoglobin and O₂, and n = 2.3.

- (a) At 37 °C, K was determined to be 53 torr. Sketch Y vs. pO₂. Mark on your graph the p50 value, where 50% of the sites are occupied (Y = 0.5).
- (b) The O₂ transport efficiency from the lung to muscle tissue is described by $\Delta Y = Y(\text{lung}) - Y(\text{tissue})$, with a larger ΔY indicating more efficient transport. It is known that pO₂(lung) = 100 torr, and pO₂(tissue) = 20 torr. At 25 °C, K decreases to 25 torr. Without doing an exact calculation, predict whether O₂ transport is more efficient at 37 °C or 25 °C. Clearly state your rationale.
- (c) It is known that 2,3-Bisphosphoglycerate (2,3-BPG) selectively binds to the T-state of the hemoglobin and reduces O₂ binding affinity. The binding pocket for 2,3-BPG is shown on Fig. 1. Propose a mutation on



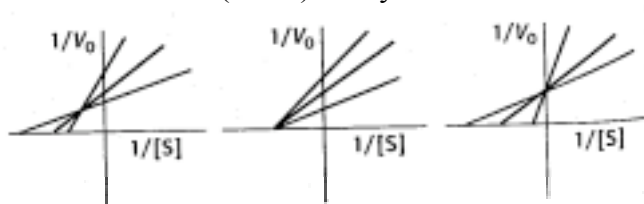
hemoglobin that will disrupt 2,3-BPG binding. Clearly state your rationale.

3. (30 pts)

- (a) Based on the steady-state assumption, derive the Michaelis-Menten (“Briggs-Haldane”) equation for $E+S \rightleftharpoons ES \rightarrow E+P$
- (b) Construct a Lineweaver-Burk plot for competitive inhibitor I showing the equation and use your plot to define V_m , K_m , and K_i .

4. (30 points)

- (a) Suppose that a mutant enzyme binds a substrate 100-fold as tightly as does the native enzyme. What is the effect of this mutation on catalytic rate if the binding of the transition state is unaffected? Why?
- (b) For a one-substrate, enzyme-catalyzed reaction, double-reciprocal plots were determined for three different enzyme concentrations. Which of the following three families of curve would you expect to be obtained (circle)? Why?

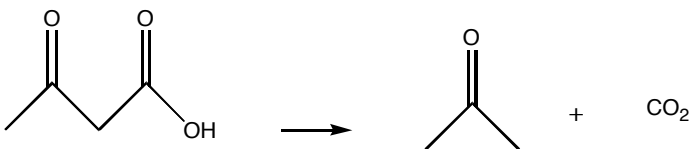


- (c) In carbonic anhydrase II, mutation of residue His 64 to Ala was expected to result in a decrease in the maximal catalytic rate. However, in buffers such as imidazole with relatively small molecular components, no rate reduction was observed. In buffers with larger molecular components, significant rate reductions were observed. Propose an explanation.
- (d) Calculate the standard redox potential for $NAD^+/NADH+H^+$ (E_0' -0.32 V) coupled to $Cyt_c (+3)$ to $Cyt_c (+2)$ (E_0' +0.22 V), the standard free-energy change $\Delta G_0'$ and then the K_{eq} for the coupled reaction. Include units.
- (e) Predict the effect of the following environmental change on the pK of a lysine amino side chain: the terminal carboxyl group of the protein is brought into close proximity.

5. (30 points)

- (a) Give one example of a class of phosphoryl transfer enzyme (name and type of reaction catalyzed)

- (b) If the bimolecular rate of carbon dioxide hydration is $0.06 \text{ M}^{-1}\text{s}^{-1}$, what would the pseudo-first order rate in water be (value and unit(s)).
- (c) Draw the structure of the trigonal-bipyramidal intermediate in attack of a water molecule on ATP at pH 7.
- (d) An enzyme E catalyzes:



How would you determine whether an imine vs. a bound metal intermediate is involved in catalysis?

- (e) According to the Table below, for the chymotrypsin substrates indicated, alkyl esters all had the same K_m and k_{cat} , but *p*-nitrophenyl had a smaller K_m (but the same k_{cat}). The amide had a much bigger K_m and much smaller k_{cat} . Explain the results briefly and qualitatively in terms of a simple multi-step catalytic scheme.

Table 3-5
Kinetic parameters for *N*-acetyltryptophanyl substrates

Generalized structure	X	K_m (M)	k_{cat} (Turnover number) [moles min^{-1} (mole enz) $^{-1}$]
	—OEt	9.7×10^{-5}	26.9
	—OMe	9.5×10^{-5}	27.7
	—OPNP	0.2×10^{-5}	30.5
	—NH ₂	500×10^{-5}	0.036

Bonus Question (5 points):

In honor of the marathon, explain what compound overall is the major source of energy for the marathoners, and why it produces a different average speed than, say, for sprinters.