Name: ________________________________ Exam 2

Imagine that you have prepared a crude lysate sample from *E.coli* cells that contains a mixture of six proteins (A through F). Below is a table of protein characteristics:

Protein	Molecular Weight (kDa)	Isoelectric Point (pI)
A	38	3.7
B	22	4.8
C	4	5.3
D	75	6.8
E	55	9.5
F	115	5.3

1. If you separate the proteins using a gel-filtration (also known as a size-exclusion) column, what order do you expect the six proteins to elute from the column?

(10)

2. If you apply the six proteins to a cation exchange column (negatively charged column resin) equilibrated to pH 5.0,

(10)

   a. Which protein or proteins will not be retained on the column?

   b. What order do you expect the protein or proteins to elute that are retained on the column as the pH of the mobile phase is slowly raised to 10.

3. If you resolve the six proteins on an SDS-PAGE gel, rank the expected result in order from high to low R_f value.

(10)

High (1)-- --Low (0)

4. Determine the specific activity in mU/mg for a β-galactosidase protein solution with a concentration equal to 0.234 mg/mL and the following activity assay results:

(10)

Activity Assay

- 3.0 mL Total Assay Volume
- 10 μL β-galactosidase protein solution added to assay
- A_{410} equal to 0.457 after 5 minutes
- ε_{410} for ONP equal to 4.8 $mM^{-1}\ cm^{-1}$
- 1 unit of activity is defined as the production of 1 mmole of ONP per minute.

Name: ______________________________ Exam 2

The following questions pertain to Yuan, Y., Byrd, C., Shen, T., Simplaceanu, V., Tam, T., and Ho, C. (2013). Role of β/δ101Gln in Regulating the Effect of Temperature and Allosteric Effectors on Oxygen Affinity in Woolly Mammoth Hemoglobin. *Biochemistry* **52:** 8888-8897.

5. The authors study Asian elephant, woolly mammoth, and mutant woolly mammoth hemoglobin. All three versions of hemoglobin have four binding sites for oxygen. Using the following data (available as a file on the desktop) and non-linear fitting in Excel, determine the P_{50} and hill coefficient (h) for each version of hemoglobin. Error calculations are not needed.

pO2 (mmHg)	Theta		
	Asian Elephant (E101)	Woolly Mammoth (Q101)	Mutant Woolly Mammoth (Q101 to K101)
0	0.00	0.00	0.00
1	0.00	0.02	0.13
2	0.03	0.11	0.40
3	0.08	0.24	0.62
4	0.15	0.37	0.75
5	0.25	0.50	0.83
6	0.34	0.60	0.88
7	0.44	0.68	0.91
8	0.52	0.75	0.93
9	0.60	0.79	0.95
10	0.66	0.83	0.96
11	0.72	0.86	0.97
12	0.76	0.88	0.97
13	0.80	0.90	0.98
14	0.83	0.91	0.98
15	0.85	0.93	0.98
16	0.87	0.94	0.98
17	0.89	0.94	0.99
18	0.90	0.95	0.99
19	0.91	0.96	0.99
20	0.92	0.96	0.99

(20)

	Asian Elephant (E101)	Woolly Mammoth (Q101)	Mutant Woolly Mammoth (Q101 to K101)
h			
P_{50}			

6. Rank the three versions of hemoglobin from strongest to weakest affinity for oxygen.

(10)

Strongest--- ---Weakest

7. The authors suggest that the residue at position 101 of the β subunit is an important determinant of oxygen binding affinity. They argue that the chemical nature of the amino acid side chain at that position alters the ratio of the T and R states of hemoglobin at equilibrium without significantly affecting the intrinsic oxygen affinity of the T or R states. If the three hemoglobin versions were considered in the MWC model, rank the model fit parameters from smallest to largest value or indicate *no difference*.

(10)

K_{site}^{T}

Smallest--- ---Largest

K_{site}^{R}

Smallest--- ---Largest

$K_{0}^{T\rightarrow R}$

Smallest--- ---Largest

8. Draw a predicted hill plot showing the Asian elephant, woolly mammoth, and mutant woolly mammoth hemoglobins following the MWC model.

(10)

9. Asian elephant hemoglobin has glutamate at position 101. Woolly mammoth hemoglobin has glutamine at position 101. A mutant wooly mammoth hemoglobin studied by the authors has lysine at position 101.

(10)

a. Draw the chemical structure of glutamate, glutamine, and lysine side chains at pH 7.4.

b. Make a list of all the types of interactions that each side chain could make with adjacent amino acid side chains within each hemoglobin structure beside your chemical structures in "a".

c. Considering your rank ordering for the oxygen affinities of these three hemoglobin versions from #6, what chemical property at position 101 seems to be attributable to the change in the ratio of the T to the R state for hemoglobin?