Biochemistry I – Problem set 4 *Due Oct 10, 2005* *Total Time required: ~70 min*

A: Problems from Campbell: Chapter 4: 4, 7, 20, 25, 26, 33

B: Submit answers to the following problems:

1. (14 points, 25 min) You are required to determine the affinity of an Fab antibody preparation for the hapten DNP. You carry out a series of equilibrium dialysis experiments by placing the Fv preparation inside a dialysis bag at a concentration of 10 μM (μM=10^{-6}M) and varying the concentration of DNP. The following data are obtained. In the table below, $[DNP]_{outside}$ refers to the equilibrium concentration of DNP outside of the dialysis bag. $[DNP]_{inside}$ refers to the total concentration of DNP inside the dialysis bag after equilibrium has been reached. This would include free DNP as well as DNP that is bound to the Fab fragment.

Experiment #	$[DNP]_{outside}$	$[DNP]_{inside}$
1	0.03 μM	1.33 μM
2	0.09 μM	3.19 μM
3	0.27 μM	5.97 μM
4	0.81 μM	8.81 μM
5	2.43 μM	11.63 μM

i) Calculate the concentration of DNP-bound Fab fragment ([ML]) inside the dialysis bag for each experiment. (2.5 pts)
ii) Using these values calculate the fractional saturation (Y) at each ligand concentration. (2.5 pts)
iii) Determine the K_D of binding using a Scatchard analysis. (5 pts)
iv) Given what you know about the structure of the Fab fragment, how many binding sites for DNP do you expect per molecule of Fab? Is your conclusion supported by the data? (2 pts)
v) How would your Scatchard plot be different if the analysis had been carried out with the intact antibody molecule? (2 pts)

2. (6 points, 10 min) The ability of hemoglobin to bind O_2 cooperatively is critical for its ability to transport O_2 from the lungs to tissues.

i) What would happen if the binding of hemoglobin to O_2 were *not* cooperative, but its affinity for O_2 were 26 Torr? Would it bind O_2 efficiently in the lungs? Would it unload O_2 efficiently in tissues? Include a *brief* description of the binding curve of this version of hemoglobin relative to that of both normal hemoglobin and myoglobin. (3 pts)
ii) What would happen if the binding of hemoglobin to O_2 were *not* cooperative but its affinity for O_2 were identical to that of myoglobin (i.e. 2.8 Torr)? Would it bind O_2 efficiently in the lungs? Would it unload O_2 efficiently in tissues? Include a *brief* description of the binding curve of this version of hemoglobin relative to that of both normal hemoglobin and myoglobin. (3 pts)

3. (6 points, 10 min) Fetal hemoglobin (HbF) contains a serine in place of the cationic histidine residue at position 143 of the □ chains of adult hemoglobin (HbA). Residue 143 faces the central cavity between the □ chains.

i) Why does 2,3 BPG bind more tightly to deoxy HbA than to deoxy HbF? (2 pts)

ii) How does the decreased affinity of HbF for 2,3 BPG affect the affinity of Hb for O_2? (2 pts)

iii) The P_{50} for HbF is 18 torr, whereas the P_{50} for HbA is 26 torr. Provide a reasonable explanation for the advantage conferred by the His to Ser change in HbF in the transfer of O_2 from maternal blood to the fetus. (2 pts)

4. (11 points, 25 min) A mutant form of hemoglobin has just been discovered and you carry out a ligand binding analysis to analyze its characterisitics. The following data are observed:

$P0_2$ (Torr)	Y	PO_2 (Torr)	Y
0.3	.002	30	.50
0.9	.005	45	.68
2.5	.014	60	.78
5	.032	75	.83
7.5	.067	100	.87
10	.11	150	.91
15	.21	200	.93

i) Using your values of Y, plot a Hill plot. Determine the average K_D value and the Hill coefficient. How do your values compare to those for the wild type protein (K_D = 26 Torr, n_h = 3)? (5 pts)

ii) Also using the Hill plot, estimate the values of K_{D1} for the mutant hemoglobin. How does it compare to the K_{D1} of wild type hemoglobin (180 Torr)? (3 pts)

iii) Based on the Hill coefficient of the mutant protein, do you expect K_{D4} of the mutant hemoglobin to be higher or lower than that of the wild type protein (K_{D4} wt = 0.1 Torr)? (3 pts)