Problem set 4 – solution key

1. (14 pts) There will probably be two sets of answers to this problem. One from students who received my email with corrections for values of [DNP]inside, and another from students who did not. The corrected problem and solution are presented first. If the student's answer does not coincide, please see below.

Experiment #	$[DNP]_{outside}$	$[DNP]_{inside}$
1	0.03 μM	1.3 μM
2	0.09 μM	3.2 μM
3	0.27 μM	6.0 μM
4	0.81 μM	8.8 μM
5	2.43 μM	11.6 μM

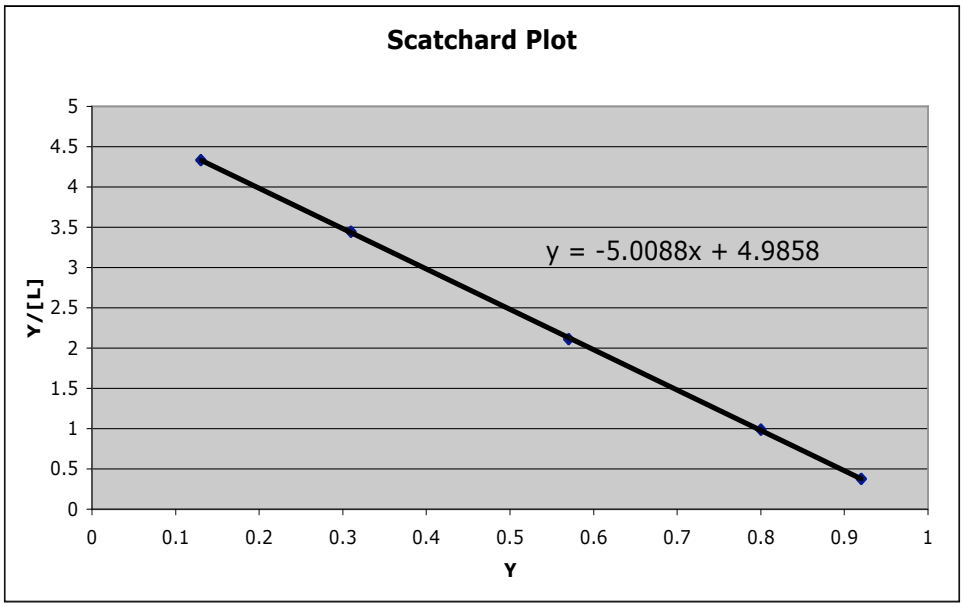
i) **(2.5 pts, 0.5 pt each)** [ML] = [L]in – [L]out:

Experiment:	1	2	3	4	5
[ML]	**1.27 μM**	**3.11 μM**	**5.73 μM**	**7.99 μM**	**9.17 μM**

ii) **(2.5 pts)** Y=[ML]/[M]total:

Experiment:	1	2	3	4	5
Y	**0.13**	**0.31**	**0.57**	**0.80**	**0.92**

iii) Scatchard Analysis: Plot Y/[L] versus Y. The slope of the resulting line should give $-1/K_D$.



Slope = $-1/K_D$, therefore $K_D = -1/\text{slope} = -1/(-5) =$ **0.2 μM (5 pts)**

Experiment #	$[DNP]_{outside}$	$[DNP]_{inside}$
1	0.03 μM	1.3 μM
2	0.09 μM	3.2 μM
3	0.27 μM	5.7 μM
4	0.81 μM	8.0 μM
5	2.43 μM	9.2 μM

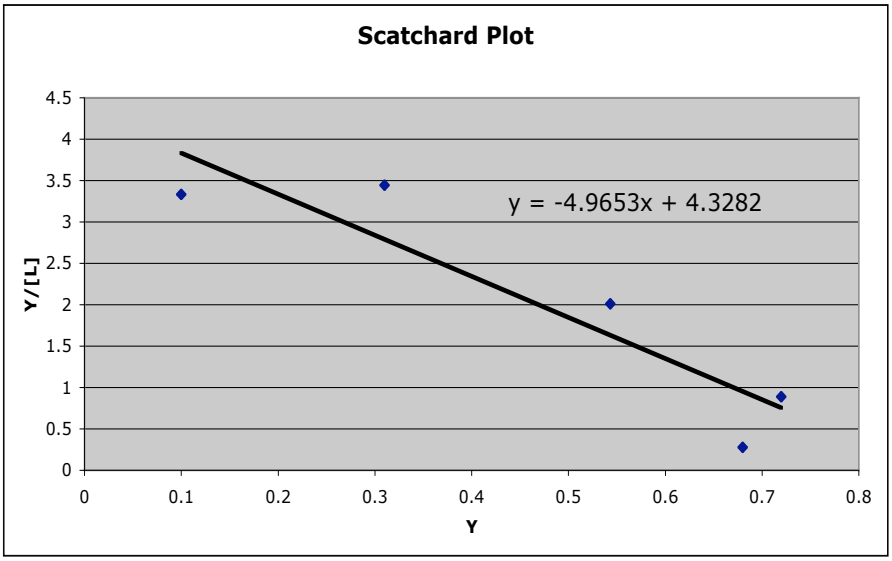
Students who used the original (incorrect) data will have gotten the following answers. Full credit should be given for the following answers.

i) **(2.5 pts, 0.5 pt each)** [ML] = [L]in – [L]out:

Experiment:	1	2	3	4	5
[ML]	**1.0 μM**	**3.11 μM**	**5.43 μM**	**7.19 μM**	**6.77 μM**

ii) **(2.5 pts)** Y=[ML]/[M]total:

Experiment:	1	2	3	4	5
Y	**0.10**	**0.31**	**0.543**	**0.72**	**0.68**



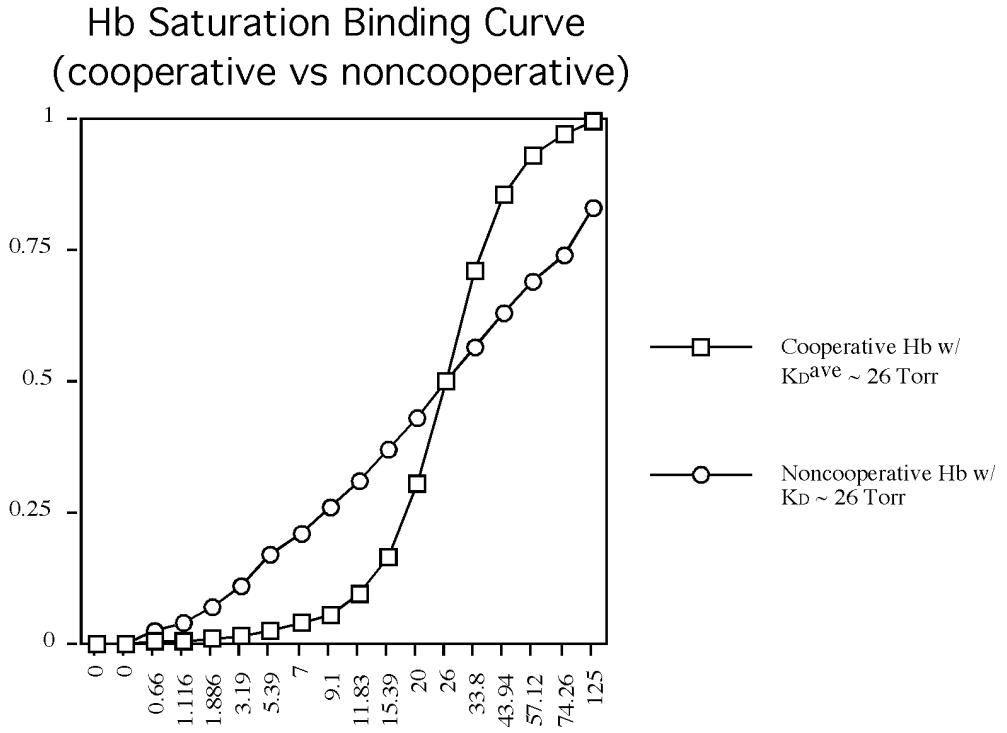
Slope = -1/K_D, therefore K_D = -1/slope = -1/(-5) = **0.2 μM (5 pts) The slope is the same for either set of data.**

iv) The Fab fragment has **one antigen-binding site per molecule**. **(1 pt)** This is supported by two observations: **1) the binding curve saturates at 1**; and 2) **the x-intercept of the Scatchard plot (Y when [L] is infinitely large) is 1**. **(1 pt)** for either answer. **If the student answered that the there is 0.7 - 0.9 binding site per molecule, they should receive full credit because the original data will have led them to such a result.**

v) **If the experiment were carried out with the intact antibody that has two antigen binding sites per molecule, $[L]_{in} - [L]_{out}$ would give the partial saturation, , and would range from 0 to 2. When plotted on a Scatchard plot, the x-int would be 2 rather than 1. (2 pts)**

2. (6 pts) i) The binding curve of this version of hemoglobin would be **hyperbolic**, unlike that of normal hemoglobin**. Its half saturation point would still be 26 torr, but it would bind less O2 in the lungs and release O2 less readily in tissues.** Overall, O_2 delivery would be much less. **(3 pts)**

[See graph below for a comparison between cooperative and non-cooperative Hb with the same K_D/K_D^{ave}. Note that at the partial pressure of the lungs (100 torr), only 75% of the non-cooperative Hb has O_2 bound. And at the partial pressure of tissues (~20 torr), the non-cooperative Hb remains 40% saturated with O_2, giving a delivery of only 35% of bound O_2. This should be compared to cooperative Hb, which has >95% of its sites bound with O_2 at 100 torr and only 30% O_2 bound at 20 torr, giving a delivery of >60% of bound O_2.] **This level of explanation is not required for full credit.**



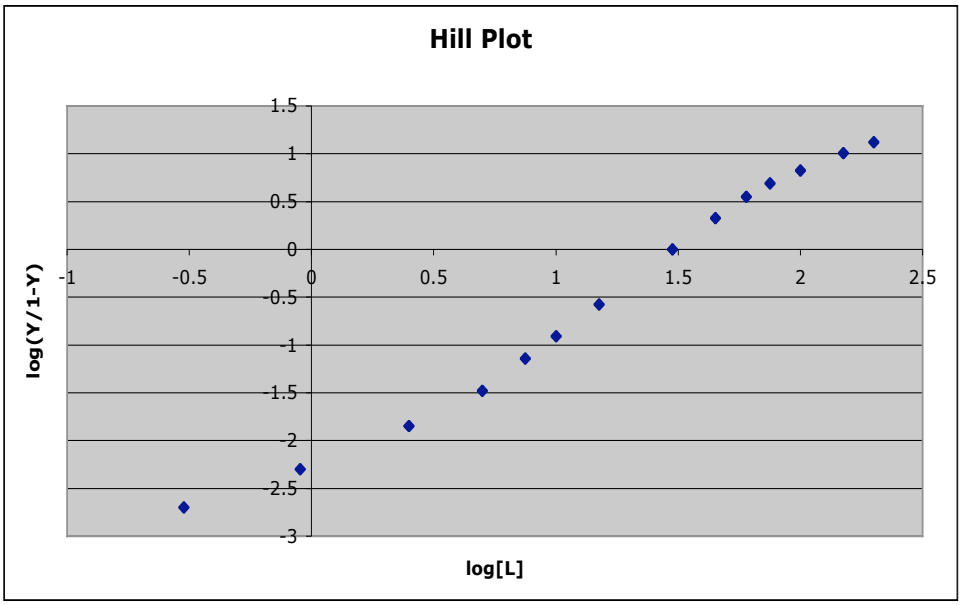
ii) The binding curve of this version of hemoglobin would be **hyperbolic and identical to that of myoglobin**. **(1.5 pts) It would bind O_2 very efficiently in the lungs but would release little if any O_2 in tissues**. **(1.5 pts)** Overall, O_2 delivery would be much less.

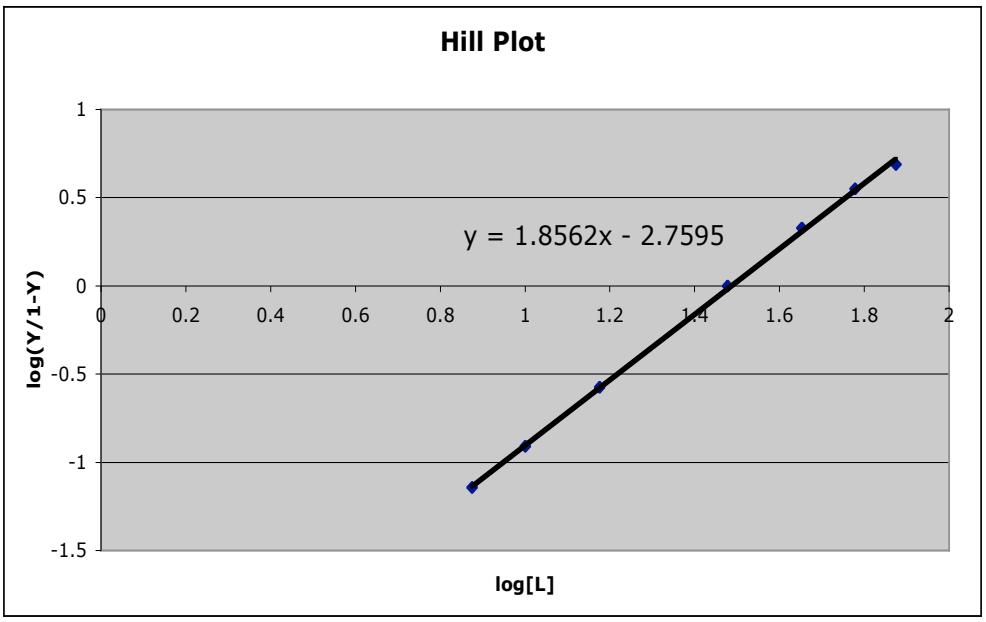
3. (6 pts) i) **2,3 BPG binds to positively charged side chains in the central cavity of deoxyhemoglobin. Since HbF lacks two of the positively charged residues, 2,3 BPG binds less tightly**. **(2 pts)**

ii) **2,3 BPG stabilizes the deoxy form of hemoglobin. Since HbF binds 2,3 BPG less tightly than does HbA, the deoxy form of HbF is less stable. Consequently, HbF has a greater affinity for O_2 at all oxygen pressures. (2 pts)**

ii) **At the oxygen pressure of tissues (20-40 torr), HbF has a greater affinity for O_2 than does HbA. Thus HbF is more likely to retain its bound O_2 until it reaches the fetus. (2 pts)**

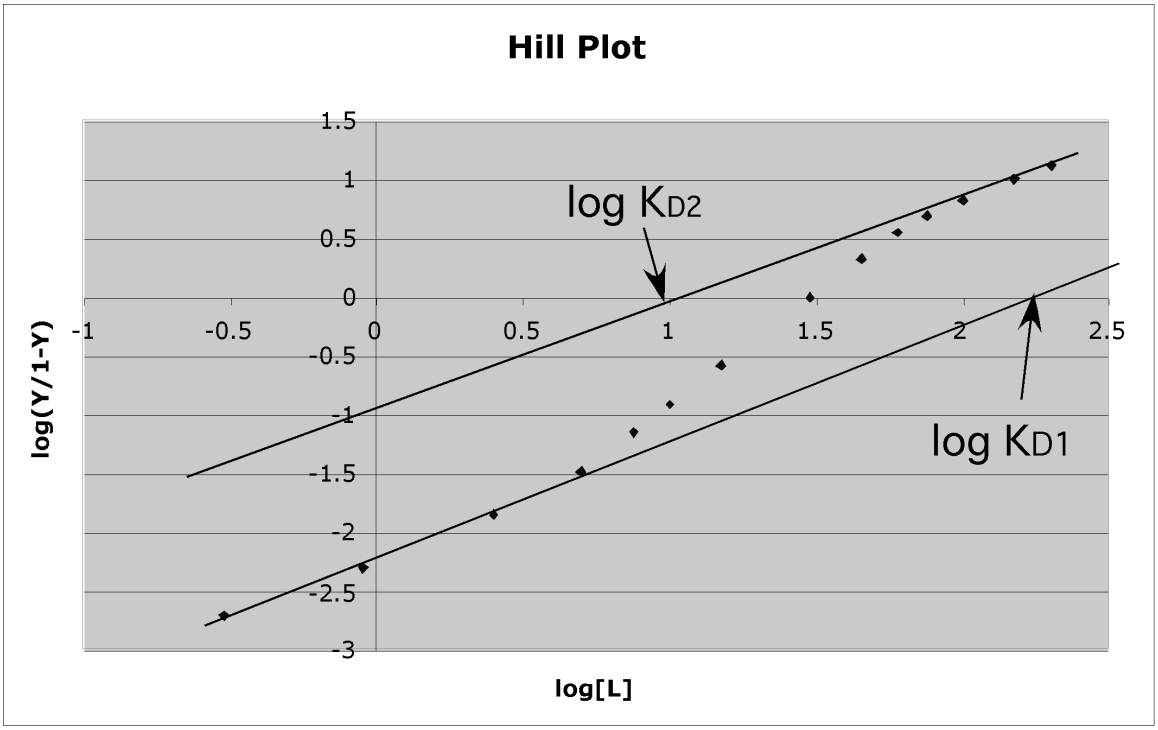
6. (11 pts)
The complete Hill plot is shown below. It exhibits positive cooperativity and is curved because it encompasses K_{D1}, K_{D2} as well as K_D^{ave}.
To determine the Hill coefficient, we need to determine the slope of the line about Y=0.5. This is shown below.





i) The slope of the line about Y=0.5 is ~1.9. **Thus the Hill coefficient for the mutant Hb, n_h, is ~1.9**. **(3 pts)** The K_D^{ave} is the ligand concentration where the line crosses the x-axis (again, when Y=0.5). The x-int is ~ 1.5. Thus log K_D^{ave} =log{1.5} and K_D^{ave}= 32 torr. **Thus the K_D^{ave} for the mutant Hb is ~ 32 torr**. (2 pts). **The mutant Hb has a slightly lower overall affinity than wt and it is also exhibits less cooperativity.**

ii) K_{D1} for the mutant Hb can be estimated by drawing a line with a slope of 1 through the points on the Hill plot at low ligand concentrations. The point at which this line intersects the x-axis is log K_{D1}. Here, log K_{D1} is ~2.25. **Thus K_{D1} is ~178 torr. (3 pts) This is almost identical to the K_{D1} of normal Hb.**



iii) Because the mutant Hb has a K_{D1} identical to that of wt Hb and yet it is is significantly less cooperative, the expectation is that the mutant Hb would have a lower K_{D2} (actually K_{D4}) than the wt Hb. (3 pts) This is clearly seen by the following: The K_{D2} **(actually K_{D4})** for the mutant Hb can be estimated by drawing a line with a slope of 1 through the points on the Hill plot at high ligand concentrations. The point at which this line intersects the x-axis is log K_{D2} **(actually K_{D4})**. Here, log K_{D2} **(actually K_{D4})** is ~1. Thus K_{D2} **(actually K_{D4})** is ~10 torr. As predicted, the difference between K_{D1} and K_{D2} **(actually K_{D4})** is less for the mutant Hb than that observed for the wt Hb.