This quiz is about protein-ligand binding. You study binding of ligand L to three proteins, A, B, and C at 25°C. You measured the concentration, [PL], of the ligand-bound form of each protein at various ligand concentrations. The total protein concentrations ($[P_{total}]$) were A: 2 μM, B: 3 μM, C: 1 μM. Your results are summarized in the table below. Using these data, answer the following questions. *Explain your assumptions*.

Protein	A	B	C
$[L_{total}]$, μM	[PL], μM	[PL], μM	[PL], μM
10	0.549	0.480	0.038
20	0.867	0.831	0.138
40	1.216	1.309	0.390
60	1.402	1.616	0.590
80	1.517	1.830	0.719
100	1.595	1.987	0.800
200	1.776	2.394	0.941
500	1.904	2.726	0.990
1000	1.951	2.857	0.996*

* I noticed that when rounding of this number, I made a typo: instead of having 0.998 I entered 0.996 – this seemingly very small difference offsets the results for the last point of protein C.

I converted [PL] into θ:

Protein	A	B	C
$[L_{total}]$, μM	θ	θ	θ
10	0.2745	0.16	0.038
20	0.4335	0.277	0.138
40	0.608	0.43633	0.39
60	0.701	0.53867	0.59
80	0.7585	0.61	0.719
100	0.7975	0.66233	0.8
200	0.888	0.798	0.941
500	0.952	0.90867	0.99
1000	0.9755	0.95233	0.996

Knowing the [PL], I calculated the free ligand concentration, [L]:

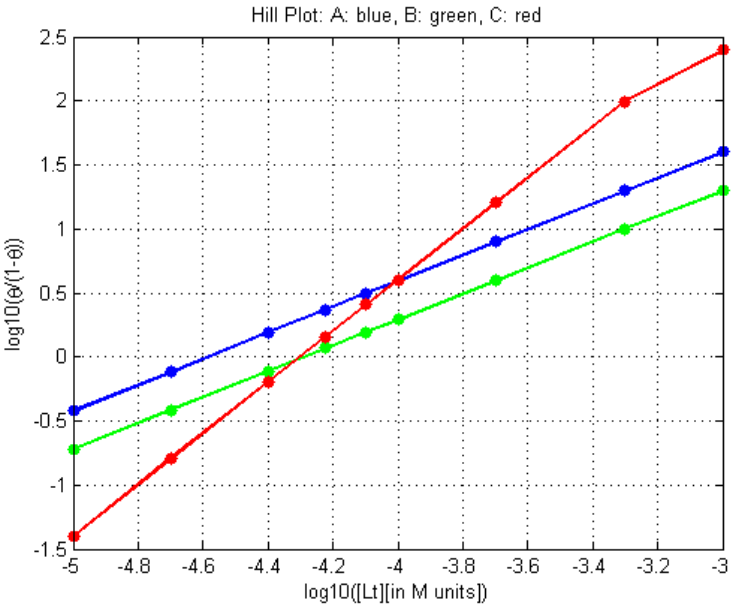
Protein	A	B	C	C*
$[L_{total}]$, μM	[L], μM	[L], μM	[L], μM	[L]*, μM
10	9.4513	9.5202	9.9615	9.9231
20	19.133	19.169	19.862	19.724
40	38.784	38.691	39.61	39.22
60	58.598	58.384	59.41	58.82
80	78.483	78.17	79.281	78.562
100	98.405	98.013	99.2	98.4
200	198.22	197.61	199.06	198.12
500	498.1	497.27	499.01	498.02
1000	998.05	997.14	999	998

*after determining that *n*=2, i.e. 2 molecules of ligand bind protein C molecule, in this column I subtracted 2x[PL] for protein C

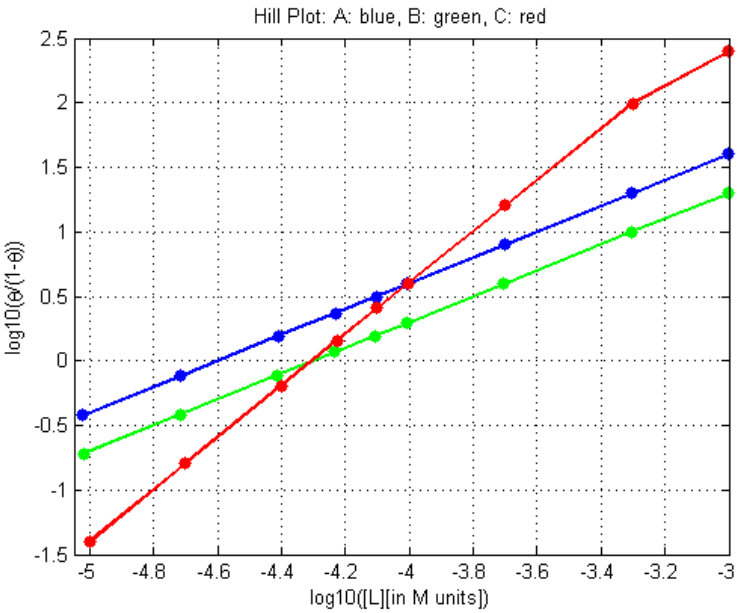
Below I provide solutions using old-fashioned way on a "graph-paper" to illustrate – and hopefully teach you -- how such problems can be solved without use of sophisticated fitting tools where you might not have full control of what the fitting program is doing.

(**1**) Is the ligand binding to each protein cooperative or non-cooperative? If the binding is cooperative, determine the Hill coefficient. Briefly (in 1-2 sentences) explain how you came to these conclusions.

Hill Plot using $[L_t]$ for [L], i.e. ***assuming* that [L] ≈ $[L_t]$**:



Hill Plot using [L] = $[L_t]$ - [PL], without the above assumption:

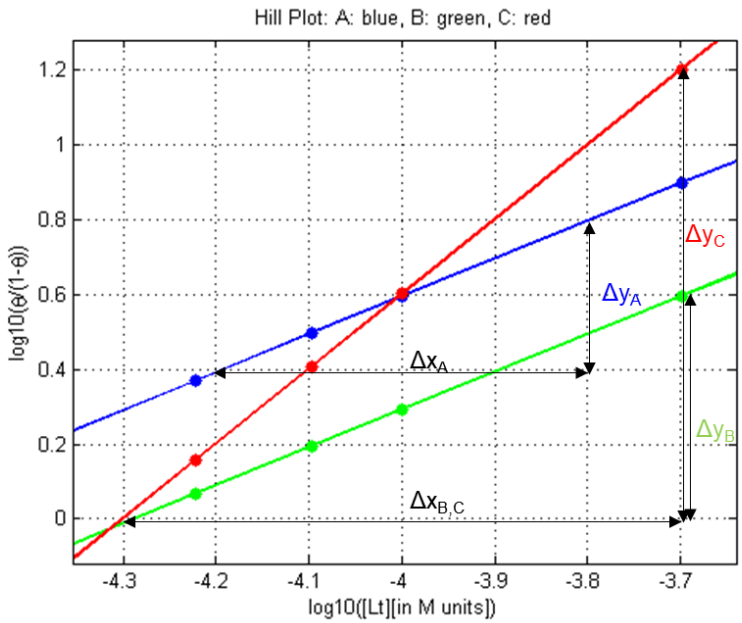


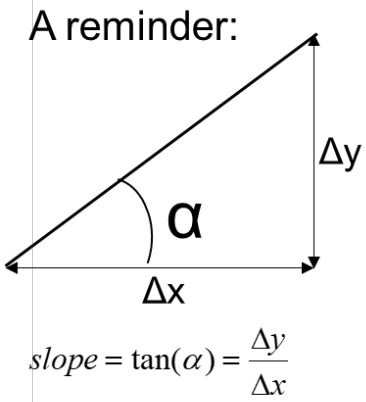
The two plots don't look much different, just a slight shift of some points along the X axis.

As you can see from the above plots, the slope (hence the *n* value) is the same for proteins A and B but is greater for protein C. Note that the values of [L] and $[L_t]$ here are in M units.

Note also that the last titration point for protein C was impacted by the round-off typo (see the footnote of the data table).

Determining/quantifying the slope in an old-fashion way, using "graph-paper" without curve/line fitting, and zooming on a part of the plot:



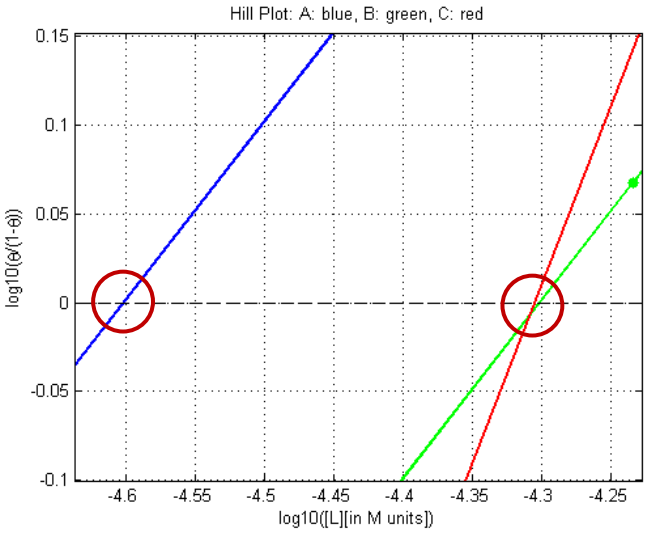


$|\Delta x_A| = 0.4$; $|\Delta y_A| = 0.4$ ➔ n = slope = $|\Delta y_A/\Delta x_A| = 1$ → non-cooperative binding
$|\Delta x_B| = 0.6$; $|\Delta y_B| = 0.6$ ➔ n = slope = $|\Delta y_B/\Delta x_B| = 1$ → non-cooperative binding
$|\Delta x_C| = 0.6$; $|\Delta y_C| = 1.2$ ➔ n = slope = $|\Delta y_C/\Delta x_C| = 2$ → cooperative binding
(you can clearly see from the plot that $\Delta y_C = 2 \times \Delta y_B$, hence twice the value of the slope and the n)

These n values agree with those I used to generate the "experimental" data.

The same results and conclusion are obtained from the Hill plots when using [L] instead of $[L_t]$ (not shown here because the plots are very similar).

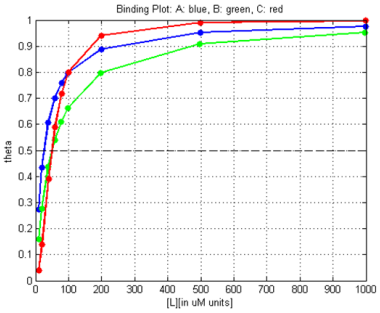
(**2**) Determine the K_d value for each protein. Briefly (in 1-2 sentences) explain how you did this.
Here I determine K_d from the intercept of the straight line in the Hill plot with the X-axis (i.e., when $\log_{10}(\theta/(1-\theta))=0$) (see the plot below). That point gives $\log_{10}([L_{0.5}])$ which can then be converted into K_d using the following equation: $K_d = [L_{0.5}]^n$.



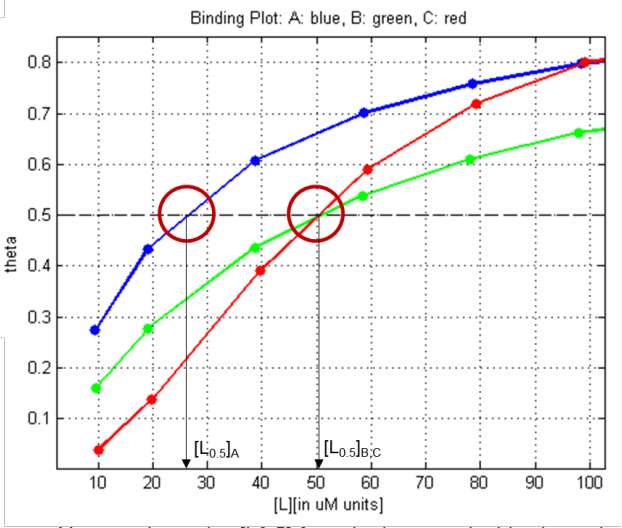
For protein A (blue line): $K_d = [L_{0.5}] \approx 10^{-4.602}$ M $\approx 25\ 10^{-6}$ M
For protein B (green line): $K_d = [L_{0.5}] \approx 10^{-4.3}$ M $\approx 50\ 10^{-6}$ M
For protein C (red line): $K_d = [L_{0.5}]^2 \approx (10^{-4.30}\ M)^2 \approx 10^{-8.60}\ M^2 \approx 25\ 10^{-10}\ M^2$
These K_d values agree with those I used to generate the "experimental" data.

The Hill plot vs $\log_{10}([L_t])$ gives slightly different values (e.g. ≈26 10^{-6} M for A and ≈51.5 10^{-6} M for B).
Note: when using ln=$\log_e$ instead of $\log_{10}$, you will need to determine $[L_{0.5}]$ as $e^{(Xintercept)}$.

You can also determine the K_d from $[L_{0.5}]$ extracted from the θ vs. [L] plot, as shown below:



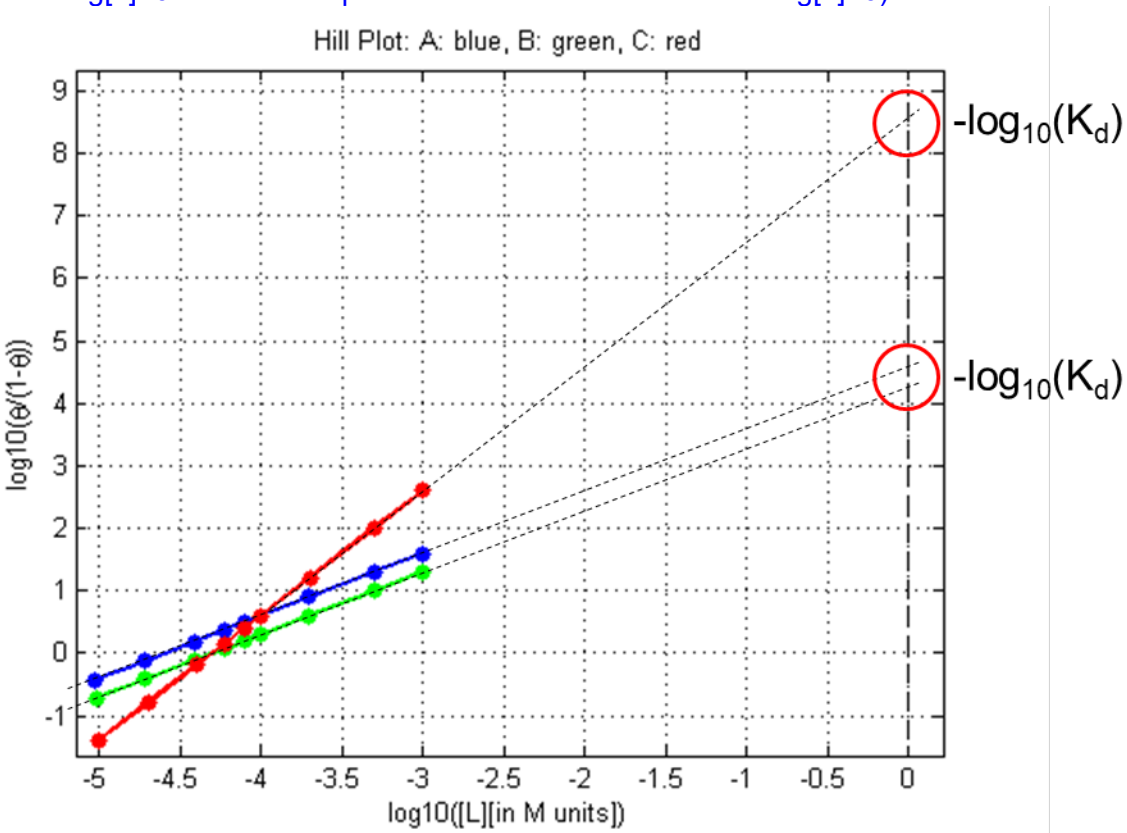
Hard to see from the above plot that for protein C the binding curve is sigmoidal, not hyperbolic. Hill plot will help here and reveal the Hill coefficient.



You can determine [L0.5] from the intercept in this plot and convert it into K_d when you know *n*.

For protein A (blue line): $K_d = [L_{0.5}] \approx 26\ \mu M = 26\ 10^{-6}$ M
For protein B (green line): $K_d = [L_{0.5}] \approx 50\ \mu M \approx 50\ 10^{-6}$ M
For protein C (red line): $K_d = [L_{0.5}]^2 \approx (50\ \mu M)^2 \approx 25\ 10^{-10}\ M^2$

Lastly, for completeness, let's determine the K_d from the intercept of the Hill plot with the Y axis (i.e. when log[L]=0. For this the plot lines need to be extended to log[L]=0):



The intercept with the Y axis gives $-\log_{10}(K_d)$, so we get the K_d value "directly" without the need to raise something to the power of *n*: $K_d = 10^{-Y\text{intercept}}$.

For protein A (blue line): intercept ≈ 4.6 → $K_d = 10^{-4.6}$ M ≈ 2.5 10^{-5} M = 25 μM
For protein B (green line): intercept ≈ 4.3 → $K_d = 10^{-4.3}$ M ≈ 5 10^{-5} M = 50 μM
For protein C (red line): intercept ≈ 8.6 → $K_d = 10^{-8.6}$ M^2 ≈ 2.5 10^{-9} M = 2500 μM^2 (the conversion of Y-intercept into K_d gives the correct value in "Molar" units but doesn't tell me the actual unites in terms of the power of M, I inferred it from knowing the *n*)

(**3**) Determine the ΔG^0 for each protein-ligand pair.

Plugging in the above determined K_d values into the equation $\Delta G^0 = RT \ln(K_d)$ gives:
ΔG^0 = -26.2 kJ/mol for protein A
ΔG^0 = -24.5 kJ/mol for protein B
ΔG^0 = -49 kJ/mol for protein C

(**4**) Which of the three proteins has the lowest affinity for the ligand? *Explain your reasoning.*

Higher K_d means weaker binding, lower affinity. Thus, protein B has lower affinity for the ligand than protein A. The K_d value for protein C is even smaller, suggesting stronger binding. However, comparing the K_d of protein C with the other two proteins could be confusing because of the different units. If you worry about this, let's use ΔG^0 as another measure of the strength of binding: it is straightforward to compare the ΔG^0 values for all 3 proteins. Higher ΔG^0 means weaker binding – lesser affinity for the ligand. From the ΔG^0 values obtained in part (3) we can conclude that protein B has the lowest affinity for the ligand – it has the highest (least negative) ΔG^0.

Note after looking at some answers: the ΔG^0 in the above equations is the change in free energy upon *binding*, not upon dissociation (!): $\Delta G^0 = RT \ln(K_d) = - RT \ln(K_a)$. Lower ΔG^0 means more favorable (stronger) association (binding) not dissociation. And, as you know:
-24.5 > -26.2 > -49.