** This examination is open book, but is to be worked on ***independently***. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is ***very important***.

## Due in LGRT 403D: 4:00pm, Monday, May 20

*Show your work for full credit. Be concise, but complete.*
*Avoid long rambling answers which indicate that you don't really understand the question.*

1. (15 points) Starting from the integrated form of the Gibbs-Helmholtz equation:

$$\frac{\Delta G_{T_2}}{T_2} - \frac{\Delta G_{T_1}}{T_1} = -\int_{T_1}^{T_2} \frac{\Delta H}{T^2} \partial T$$

Derive an expression for the temperature dependence of the equilibrium constant. In other words, solve for the equilibrium constant ($K_T$) at temperature T, given the known equilibrium constant ($K_{T_0}$) at a reference temperature $T_0$.

What assumption must you make (in order to make this a simple, 3-step derivation worthy of only 15 points)?

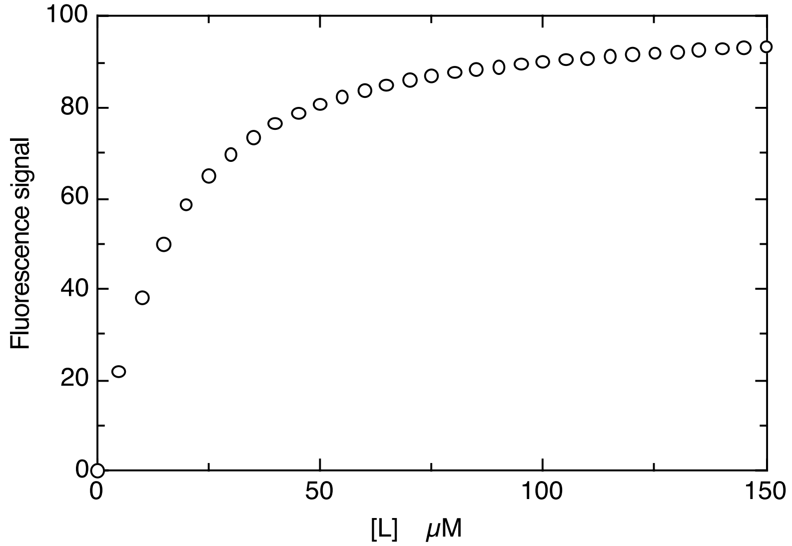
In order to evaluate the integral on the right side of the equation, we must assume that ΔH is independent of temperature over the temperature range from $T_o$ to T.

$$\frac{-RT\ln K_T}{T} - \frac{-RT_o \ln K_{T_o}}{T_o} = -\Delta H \int_{T_o}^{T} \frac{\partial T}{T^2} = -\Delta H\left[-\left(\frac{1}{T} - \frac{1}{T_o}\right)\right]$$

$$-R\ln K_T + R\ln K_{T_o} = \Delta H\left(\frac{1}{T} - \frac{1}{T_o}\right)$$

$$K_T = K_{T_o} e^{-\frac{\Delta H}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right)}$$

2. (25 points) You have collected the (beautiful quality) fluorescence titration shown below.



Conditions are as follows: $[\mathrm{Enz}]_{\mathrm{Total}} = 10.0\ \mu\mathrm{M}$ T = 25° C

Think about how you could derive the dissociation constant, $K_d$, from the plot above. The "casual biochemist" will tell you that $K_d$ is the concentration (15.0 μM) at which the fluorescence change is half-maximal. Having taken Chem 728, you tell him that this isn't correct.

a) Explain why you are so critical of the casual biochemist.

That way of calculating $K_d$ assumes ligand in large excess over protein. In this case, $[\mathrm{Enz}]_{tot} = 10\ \mu\mathrm{M}$ (by definition). At half saturation, $[L]_{tot} = 15\ \mu\mathrm{M}$. This is NOT large excess…

Many of you noted that we need to know the number of binding sites on the protein. This is true, but is not enough. Even if we know it's one site, the answer is wrong.

b) Derive the correct equation relating the "half-maximal fluorescence signal" in terms of the true dissociation constant $K_d$.

MY answer is below, but Travis came up with a much simpler, more elegant solution. Congratulations to Travis!! (Abdalin also had one better than mine, congratulations!). Here's Travis' answer in **two** lines:

$$K_d = \frac{[P][L]}{[PL]} \quad \text{At half - saturation, } [\mathrm{P}] = [PL] = \frac{1}{2}[P]_{tot}$$

$$K_d = \frac{\frac{1}{2}[P]_{tot}\left([L]_{tot}^{half\ \max} - \frac{1}{2}[P]_{tot}\right)}{\frac{1}{2}[P]_{tot}} = [L]_{tot}^{half\ \max} - \frac{1}{2}[P]_{tot}$$

**My, much uglier answer**: The equation that we need, we talked about in class. It is:

$$[PL]=\frac{\left([P]_{tot}+[L]_{tot}+K\right)\pm\sqrt{\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}}}{2}$$

We want to know what concentration of L at which $[PL]=\frac{1}{2}[P]_{tot}$

$$[PL]=\frac{1}{2}[P]_{tot}=\frac{\left([P]_{tot}+[L]_{tot}+K\right)-\sqrt{\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}}}{2}$$

$$[P]_{tot}=\left([P]_{tot}+[L]_{tot}+K\right)-\sqrt{\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}}$$

$$[L]_{tot}+K=\sqrt{\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}}$$

$$\left([L]_{tot}+K\right)^{2}=\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}$$

$$[L]^{2}+2[L]_{tot}K+K^{2}=[P]_{tot}^{2}+[L]_{tot}^{2}+K^{2}+2[P]_{tot}[L]_{tot}+2[L]_{tot}K+2[P]_{tot}K-4[P]_{tot}[L]_{tot}$$

$$0=[P]_{tot}^{2}+2[P]_{tot}[L]_{tot}+2[P]_{tot}K-4[P]_{tot}[L]_{tot}$$

$$0=[P]_{tot}^{2}-2[P]_{tot}[L]_{tot}+2[P]_{tot}K=[P]_{tot}-2[L]_{tot}+2K$$

$$[L]_{tot}=K+\frac{1}{2}[P]_{tot}$$

Therefore $K=[L]_{tot}-\frac{1}{2}[P]_{tot}=15.0\mu M-\frac{10\mu M}{2}=10\mu M$

In case you didn't have the starting equation from lecture, it's not hard to derive, as we did in class.

$$[P]_{tot}=[P]+[PL] \qquad [L]_{tot}=[L]+[PL] \qquad K=\frac{[P][L]}{[PL]}=\frac{\left([P]_{tot}-[PL]\right)\left([L]_{tot}-[PL]\right)}{[PL]}$$

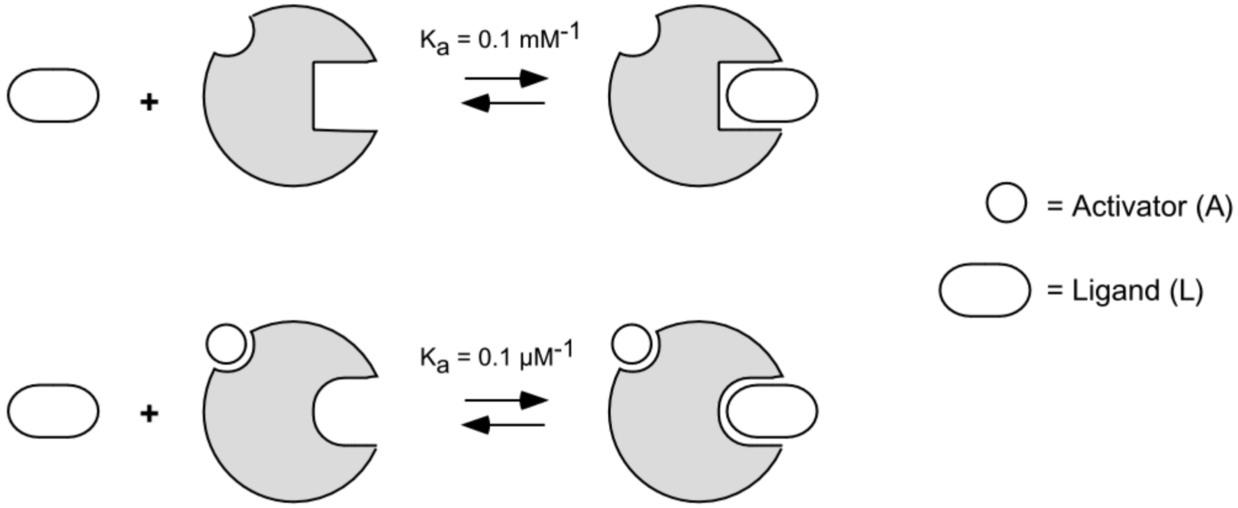
$$K[PL]=\left([P]_{tot}-[PL]\right)\left([L]_{tot}-[PL]\right)=[P]_{tot}[L]_{tot}+[PL]^{2}-[PL][L]_{tot}-[PL][P]_{tot}$$

$$0=[PL]^{2}-\left([P]_{tot}+[L]_{tot}+K\right)[PL]+[P]_{tot}[L]_{tot}$$

Quadratic Equation:

$$[PL]=\frac{\left([P]_{tot}+[L]_{tot}+K\right)\pm\sqrt{\left([P]_{tot}+[L]_{tot}+K\right)^{2}-4[P]_{tot}[L]_{tot}}}{2}$$

3. (15 points) Consider a protein (P) which binds ligand (L). In the absence of a bound activator, the association constant for L binding to protein is 0.1 $\text{mM}^{-1}$. In the presence of bound activator, the association constant for L binding to protein is 0.1 $\mu\text{M}^{-1}$.



Identify the following statements as true or false:

| | | |
|---|---|---|
| True | **False** | Binding of activator need not be effected by the presence of bound ligand. |
| **True** | False | Binding of activator is increased1000-fold by the presence of bound ligand |
| True | **False** | Binding of activator is decreased1000-fold by the presence of bound ligand. |

Explain your answers.

The illustration above was intentionally designed to remind you of a thermodynamic cycle – draw equilibria top to bottom on the left and right sides to show activator binding in the absence and presence of ligand. The thermodynamic cycle can be used to argue the above.

Alternatively, using just math, consider the following:

$$K_{a_1} = \frac{[PL]}{[P][L]} = 0.1mM^{-1} = 0.1\frac{1}{mM}\frac{10^3 mM}{M} = 10^2 M^{-1}$$

$$K_{a_2} = \frac{[PAL]}{[PA][L]} = 0.1\mu M^{-1} = 0.1\frac{1}{\mu M}\frac{10^6 \mu M}{M} = 10^5 M^{-1}$$

$$K_{a_3} = \frac{[PA]}{[P][A]} \qquad K_{a_4} = \frac{[PLA]}{[PL][A]}$$

Now, the 1st two equations can be manipulated to yield a relationship between the 2nd two.

$$\frac{K_{a_1}}{K_{a_2}} = \frac{[PL]}{[P][L]}\,\frac{[PA][L]}{[PLA]} = \frac{[PL]}{[P]}\,\frac{[PA]}{[PLA]}\,\frac{[A]}{[A]} = \frac{[PA]}{[P][A]}\,\frac{[PL][A]}{[PLA]} = \frac{K_{a_3}}{K_{a_4}}$$

$$\frac{K_{a_1}}{K_{a_2}} = \frac{K_{a_3}}{K_{a_4}} = \frac{10^2 M^{-1}}{10^5 M^{-1}} = 10^{-3}$$

$$K_{a_4} = 10^3 K_{a_3}$$

In other words, activator binding in the presence of L MUST be EXACTLY 1000 times greater than activator binding in the absence of L.

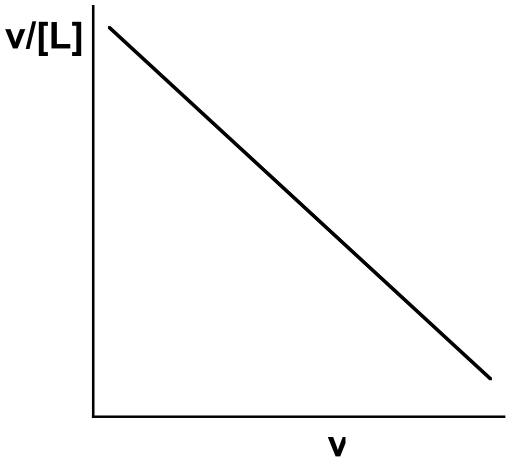
**General corollary:**

If binding of A to P helps cooperatively with binding of B to P, then

binding of B to P helps cooperatively with binding of A to P.

4. (15 points) Examine the plots below and indicate what sort of behavior each *might* show. Check as many boxes as are appropriate.

- ■ Single site
- ■ Multiple, identical independent sites
- ☐ Multiple classes of independent sites
- ☐ Multiple identical sites with positive cooperativity
- ☐ Multiple identical sites with negative cooperativity



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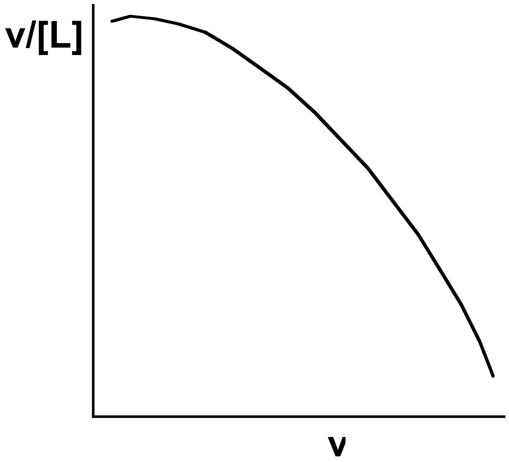

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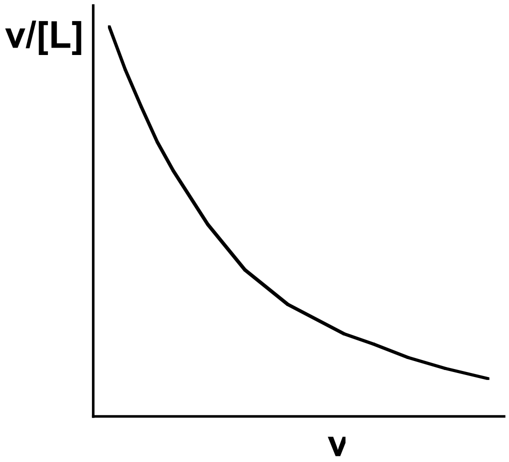

5. (30 points) The following equation shows the temperature dependency of ΔG for a reaction, assuming zero change in heat capacity.

$$\Delta G(T) = \Delta H - T\Delta S$$

a) Derive a new equation for ΔG(T) assuming a non-zero ΔCp (but assuming that ΔCp is constant, independent of temperature). Specifically, assume that you know $\Delta H(T_1)$ and $\Delta S(T_1)$, and ΔCp. Calculate ΔG at a new temperature $T_2$.

$$\Delta G(T_2) = \Delta H(T_2) - T_2\Delta S(T_2)$$

$$\int_{T_1}^{T_2} \partial\Delta H = \int_{T_1}^{T_2} \Delta C_p \partial T \qquad \Delta \mathrm{H}_{\mathrm{T}_2} - \Delta \mathrm{H}_{\mathrm{T}_1} = \Delta C_p(T_2 - T_1) \qquad \Delta \mathrm{H}_{\mathrm{T}_2} = \Delta \mathrm{H}_{\mathrm{T}_1} + \Delta C_p(T_2 - T_1)$$

Similarly,

$$\int_{T_1}^{T_2} \partial\Delta S = \int_{T_1}^{T_2} \Delta C_p \frac{\partial T}{T} \qquad \Delta \mathrm{S}_{\mathrm{T}_2} - \Delta \mathrm{S}_{\mathrm{T}_1} = \Delta C_p \ln\frac{T_2}{T_1} \qquad \Delta \mathrm{S}_{\mathrm{T}2} = \Delta \mathrm{S}_{\mathrm{T}_1} + \Delta C_p \ln\frac{T_2}{T_1}$$

and

$$\Delta G_{T_2} = \Delta H_{T_2} - T\Delta S_{T_2}$$

$$\Delta G_{T_2} = \left[\Delta H_{T_1} + \Delta C_p(T_2 - T_1)\right] - T_2\left(\Delta S_{T_1} + \Delta C_p \ln\frac{T_2}{T_1}\right)$$

$$\Delta G_{T_2} = \Delta H_{T_1} - T_2\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

b) Another way of thinking about the temperature dependence of ΔG is to calculate ΔΔG = ΔG(T2) - ΔG(T1). Use the above to derive ΔΔG in terms of ΔG(T1), ΔCp and ΔS(T1).

$$\Delta G_{T_2} = \Delta H_{T_1} - T_2\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta G_{T_2} = \Delta H_{T_1} - T_2\Delta S_{T_1} + T_1\Delta S_{T_1} - T_1\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta G_{T_2} = \Delta H_{T_1} - T_1\Delta S_{T_1} - T_2\Delta S_{T_1} + T_1\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta G_{T_2} = \Delta G_{T_1} - (T_2 - T_1)\Delta S_{T_1} + \Delta C_p(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta G_{T_2} = \Delta G_{T_1} + \left(\Delta C_p - \Delta S_{T_1}\right)(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta\Delta G = \Delta G_{T_2} - \Delta G_{T_1} = \left(\Delta C_p - \Delta S_{T_1}\right)(T_2 - T_1) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

c) Our lecture notes reflect thinking of the past decade that melting of duplex DNA has a “near-zero” change in heat capacity and therefore ΔH and ΔS can be considered to be independent of temperature. More recently, Bloomfield et al, presented arguments to the contrary. Specifically, for a particular oligonucleotide at its melting temperature (72°C), the following parameters were determined:

$$\Delta C_p = 65.3\frac{cal}{mol\ K} \qquad \Delta S = 24.9\frac{cal}{mol\ K}$$

Use the equation you derived in (b) to argue that this value of ΔCp is “not negligible.”

In a typical use of this approach, we would measure the thermodynamics near the melting temperature for the duplex, 72°C. We might want to then take those parameters and calculate back to the thermodynamics of DNA melting at room temperature (T=25°C). Let’s calculate ΔΔG. ($T_1$=345K, $T_2$=298K) we have.

$$\Delta\Delta G = \left(\Delta C_p - \Delta S_{T_1}\right)\left(T_2 - T_1\right) - T_2\Delta C_p \ln\frac{T_2}{T_1}$$

$$\Delta\Delta G = (65.3 - 24.9)\left(\frac{cal}{mol\ K}\right)\left(T_2 - T_1\right) - T_2\left(65.3\frac{cal}{mol\ K}\right)\ln\frac{T_2}{T_1}$$

$$\Delta\Delta G = \left(40.4\frac{cal}{mol\ K}\right)\left(T_2 - T_1\right) - T_2\left(65.3\frac{cal}{mol\ K}\right)\ln\frac{T_2}{T_1}$$

$$\Delta\Delta G = \left(40.4\frac{cal}{mol\ K}\right)(298K - 345K) - (298K)\left(65.3\frac{cal}{mol\ K}\right)\ln\frac{298K}{345K}$$

$$\Delta\Delta G = \left(40.4\frac{cal}{mol\ K}\right)(-47K) - (-43.6K)\left(65.3\frac{cal}{mol\ K}\right)$$

$$\Delta\Delta G = \left(-1899\frac{cal}{mol}\right) + \left(2850\frac{cal}{mol}\right) = 951\frac{cal}{mol} = 0.95\frac{kcal}{mol}$$

Assuming zero ΔCp and using the value of ΔH from part (d) below,

$$\Delta G = \Delta H - T\Delta S = 8.59\frac{kcal}{mol} - (298K)\left(0.0249\frac{kcal}{mol\ K}\right) = 8.59\frac{kcal}{mol} - 7.42\frac{kcal}{mol} = 1.17\frac{kcal}{mol}$$

0.95 is significantly different from 1.17

d) Use the equation from part (b) to calculate the value for ΔH at 72°C.

$$\Delta G = \Delta H - T\Delta S = 0$$

$$\Delta H = T\Delta S = (345K)\left(24.9\frac{cal}{mol\ K}\right) = (345K)\left(24.9\frac{cal}{mol\ K}\right) = 8.59\frac{kcal}{mol}$$