** 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.*

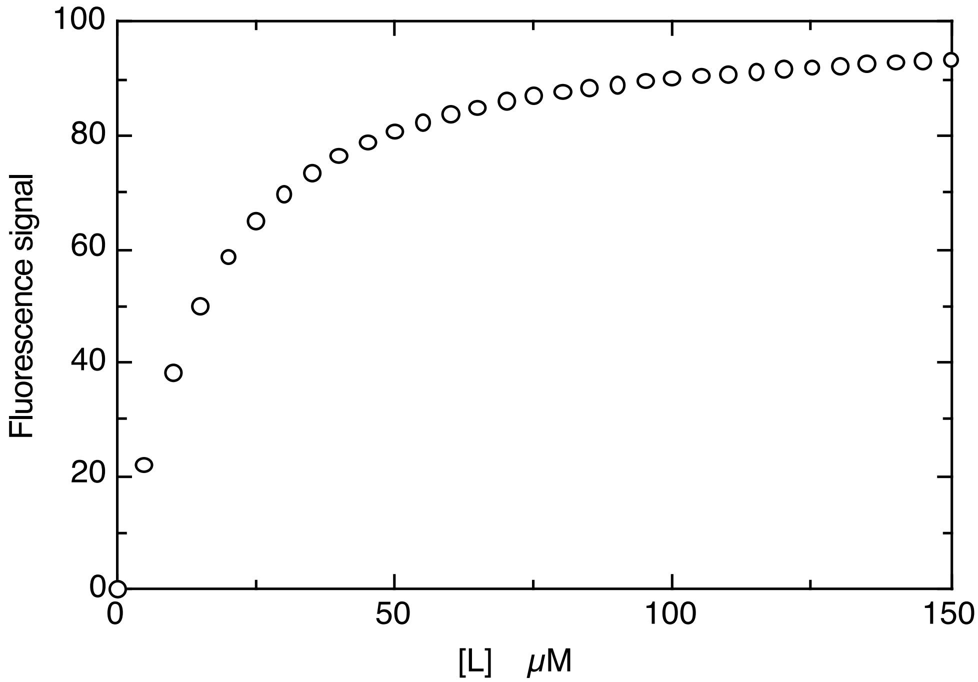
1. (15 points) Starting from the integrated form of the Gibbs-Helmholtz equation:

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

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)?

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



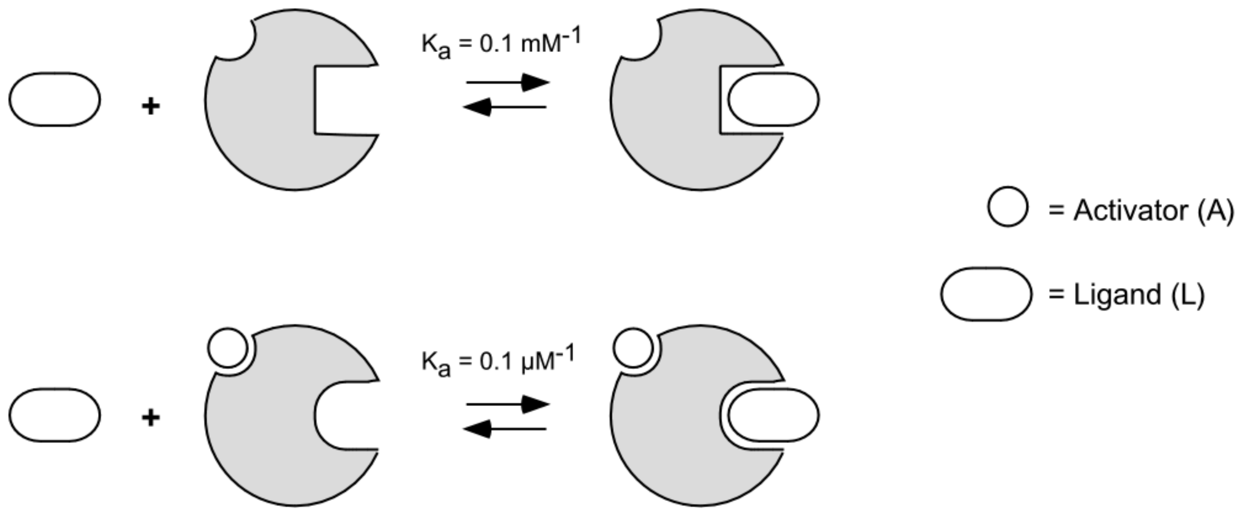

Conditions are as follows: $[Enz]_{Total} = 10.0\ \mu M$ T = 25° C

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 $\mu$M) 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.

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

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 $mM^{-1}$. In the presence of bound activator, the association constant for L binding to protein is 0.1 $\mu M^{-1}$.




Identify the following statements as true or false:

True False Binding of activator need not be effected by the presence of bound ligand.

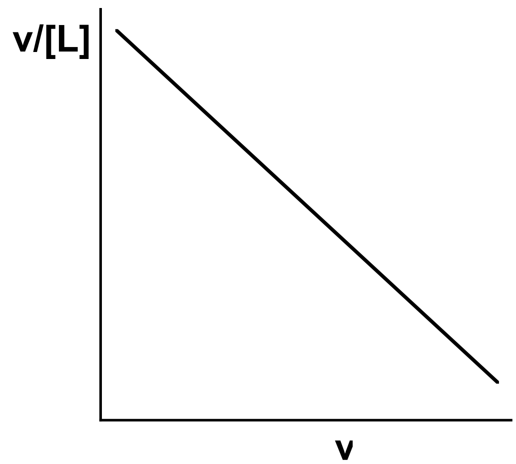
True False Binding of activator is increased1000-fold by the presence of bound ligand

True False Binding of activator is decreased1000-fold by the presence of bound ligand.

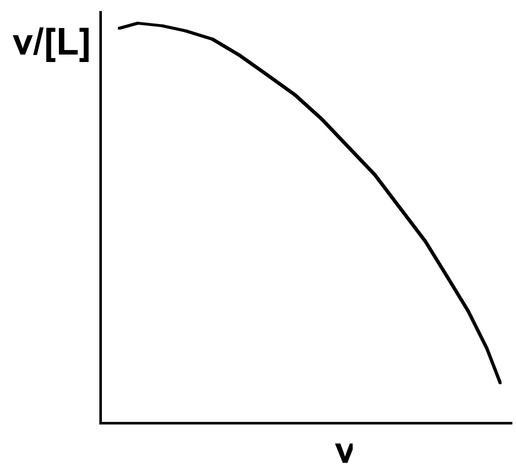
Explain your answers.

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

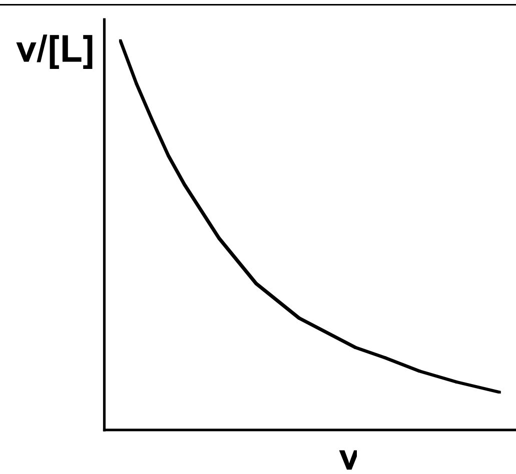
- ❑ Single site
- ❑ Multiple, identical independent sites
- ❑ Multiple classes of independent sites
- ❑ Multiple identicalsites with positive cooperativity
- ❑ Multiple identicalsites with negative cooperativity




- ❑ Single site
- ❑ Multiple, identical independent sites
- ❑ Multiple classes of independent sites
- ❑ Multiple identicalsites with positive cooperativity
- ❑ Multiple identicalsites with negative cooperativity




- ❑ Single site
- ❑ Multiple, identical independent sites
- ❑ Multiple classes of independent sites
- ❑ Multiple identicalsites with positive cooperativity
- ❑ Multiple identicalsites with negative cooperativity



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

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

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

b) Another way of thinking about the temperature dependence of $\Delta G$ is to calculate $\Delta\Delta G = \Delta G(T2) - \Delta G(T1)$. Use the above to derive $\Delta\Delta G$ in terms of $\Delta G(T1)$, $\Delta Cp$ and $\Delta S(T1)$.

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 $\Delta H$ and $\Delta S$ 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 $\Delta Cp$ is "not negligible."

d) Use the equation from part (b) to calculate the value for $\Delta H$ at 72°C.