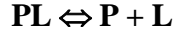


**Chem 4540 Enzymology Winter 2005**  
**Problem Set #4**

1. In an equilibrium dialysis experiment it was found that the concentrations of the free substrate analogue, bound analogue, and enzyme are  $1.2 \times 10^{-5}$  M,  $5.4 \times 10^{-6}$  M, and  $4.9 \times 10^{-6}$  M, respectively. Calculate the dissociation constant for the reaction:



Assume that there is one binding site per enzyme molecule.

2. The protease, thermolysin, binds calcium ion to form a protein:metal 1:1 complex. The following data were obtained in a typical binding experiment.

Total $\text{Ca}^{2+}$ ( $\mu\text{M}$ )	24	60	120	180	240	350	480
Bound $\text{Ca}^{2+}$ ( $\mu\text{M}$ )	13.9	31.2	51.2	63.4	70.8	75.2	78.4

Determine by graphical method the dissociation constant of the  $\text{Ca}^{2+}$ -thermolysin complex. The protein concentration was kept at  $92 \mu\text{M}$  for each run.

3. The enzyme, exotoxin A from *Pseudomonas aeruginosa*, acts on two physiological substrates,  $\text{NAD}^+$  and eukaryotic elongation factor-2 (a protein). When  $\text{NAD}^+$  alone binds to the enzyme it quenches the intrinsic tryptophan fluorescence of the protein. This observation can be used to measure the binding constant for the enzyme-toxin with  $\text{NAD}^+$ . The data for a particular experiment involving the titration of the enzyme with  $\text{NAD}^+$  are shown below:

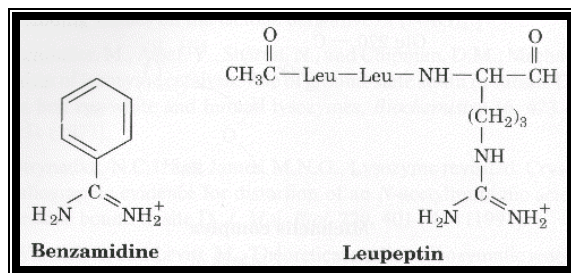
The toxin-enzyme is a monomer and binds one molecule of  $\text{NAD}^+$  per enzyme molecule. Plot the binding data and also construct a linear transformation plot of the data. Then calculate the  $K_d$  for the interaction of  $\text{NAD}^+$  with the toxin-enzyme (note: these are real data from the Merrill lab).

$[\text{NAD}^+]$ $\mu\text{M}$	Relative Fluorescence Intensity
0	8.07
10	6.77025
20	5.88088
30	5.2318
40	4.82188
50	4.39344
100	3.2232
150	2.67003
200	2.4648
250	1.9261
300	1.83874
400	1.60735
500	1.4588
750	1.21452
1000	1.09304

4. The exotoxin A enzyme also binds the protein, elongation factor-2, with a 1:1 stoichiometry. A binding assay was devised where a fluorescence donor chromophore was added to the enzyme and a fluorescence acceptor chromophore was added to the elongation factor-2 protein. When the two proteins associate with one another then fluorescence resonance energy transfer occurs between the donor and acceptor chromophores. The data below were recorded from one experiment involving the titration of exotoxin A with elongation factor-2 protein (EF-2). Plot the binding data and also a linear transformation of the data. Then calculate the dissociation constant for this protein-protein interaction (these are also real data from the Merrill lab).

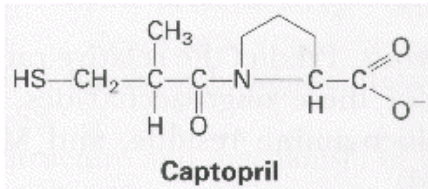
[EF-2] nM	Relative $\Delta FI$
0	0
190.1	0.21
375	0.36
554.8	0.4
900	0.54
1687.5	0.7
2382.4	0.82
3000	0.94
3552.6	0.97
4050	1

5. Benzamidine ( $K_i = 1.8 \times 10^{-5} \text{ M}$ ) and leupeptin ( $K_i = 3.8 \times 10^{-7} \text{ M}$ ) are both specific competitive inhibitors of trypsin. Explain their mechanisms of inhibition. Design leupeptin analogues that inhibit chymotrypsin and elastase.



6. Tofu (bean curd) is a high protein soybean product that is widely consumed in the Far East and is becoming more popular in North America. It is prepared in such a way as to remove the trypsin inhibitor present in soybeans. Explain the reason for this treatment.
7. Most drugs used in the management of disease, such as those that inhibit HIV-1 protease are members of what class of enzyme inhibitors? Give the hallmark of this type of inhibition, and think of one advantage and one disadvantage to the use of such compounds as therapeutic agents.

8. Angiotensin II, an octapeptide formed by the kidneys, raises blood pressure. This hormone is generated by the action of two proteolytic enzymes. First, angiotensinogen, a 14-residue peptide, is cleaved by renin to yield angiotensin I, a decapeptide. Then, the last two residues of angiotensin I are removed in a single step by the hydrolytic action of angiotensin converting enzyme (ACE) to form angiotensin II. This hormone rapidly elevates blood pressure by constricting arterioles. ACE is a zinc-containing protease that is mechanistically similar to carboxypeptidase A except it cleaves the penultimate rather than the last peptide bond. Captopril, a potent inhibitor ( $K_i = 0.2 \text{ nM}$ ) of ACE, is widely used to treat hypertension. How might captopril inhibit ACE?



9. Histidine 134 in the active site of aspartate transcarbamoylase is thought to be important in stabilizing the transition state of the bound substrates by interacting with a negative charge on a carbonyl oxygen of the substrate. Predict the pH dependence of the catalytic rate, assuming that this interaction is essential and dominates the pH-activity profile of the enzyme.