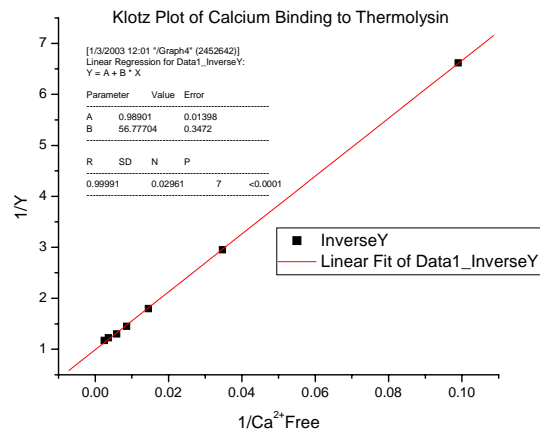
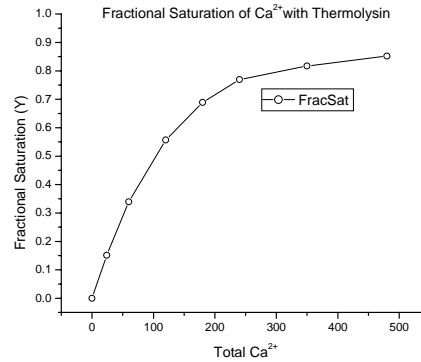
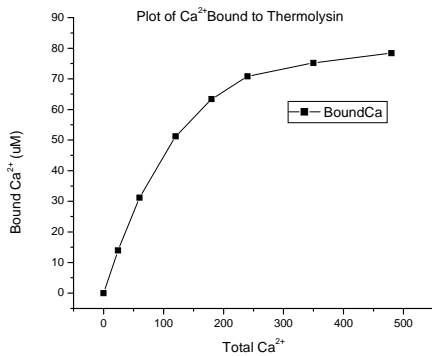


Chem 4540 Enzymology Winter 2005
Problem Set #4 ANSWERS

- Free substrate analogue = $1.2 \times 10^{-5}M$; bound analogue = $5.4 \times 10^{-6}M$; [enzyme] = $4.9 \times 10^{-6}M$. Since the binding is one site per enzyme then saturation = $4.9 \times 10^{-6}M$.
For $PL \rightleftharpoons P + L$ the $K_d = [P][L]/[PL]$
 $K_d = (4.9 \times 10^{-6}M)(1.2 \times 10^{-5}M)/(5.4 \times 10^{-6}M) = 1.08 \times 10^{-5}M$.
- Since the Ca^{2+} ligand binds the enzyme thermolysin at a 1:1 ratio then saturation for $Ca^{2+} = [thermolysin] = 92 \mu M$ bound. The fractional saturation (Y) then is simply the ratio of Bound $Ca^{2+} (\mu M)/92 \mu M$.

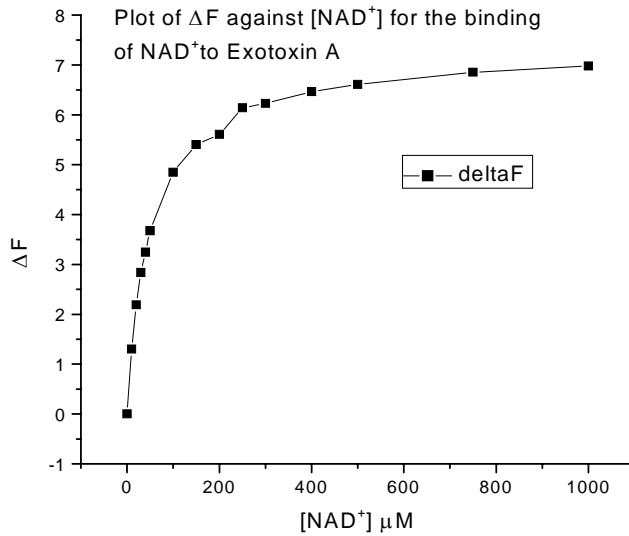
Total Ca (μM)	Bound Ca (μM)	Y	$1/Ca_{free} (\mu M^{-1})$	$1/Y$
0	0	0	-	-
24	13.9	0.1511	0.0990	6.6187
60	31.2	0.3391	0.0347	2.9487
120	51.2	0.5565	0.0145	1.7969
180	63.4	0.6891	0.00858	1.4511
240	70.8	0.7696	0.00591	1.2994
350	75.2	0.8174	0.00364	1.2234
480	78.4	0.8522	0.0025	1.1735

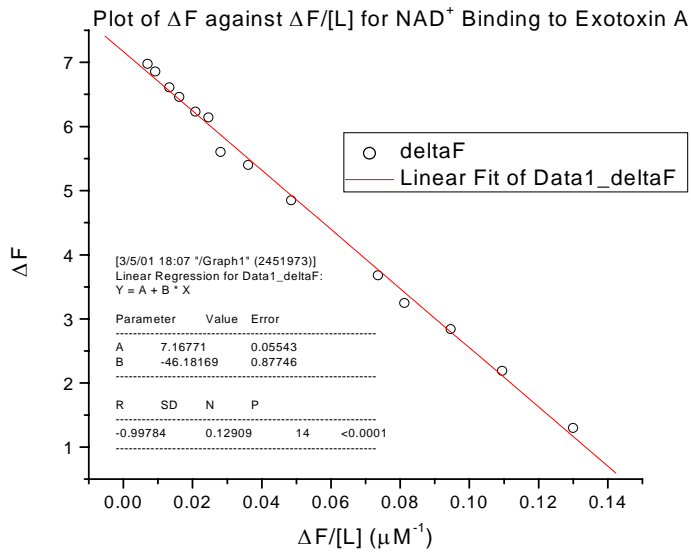


1/n = 0.989; n = 1.01; K/n = slope = 56.78 μM ; K/1.01 = 56.78 μM ;
 K = 56.78 μM * 1.01 = 57.3 μM .

3.

[NAD ⁺] μM	Rel Fluor Intensity	delta FI	delta F/[NAD+] (μM^{-1})
0	8.07	0	#DIV/0!
10	6.77025	1.29975	0.129975
20	5.88088	2.18912	0.109456
30	5.2318	2.8382	0.094606667
40	4.82188	3.24812	0.081203
50	4.39344	3.67656	0.0735312
100	3.2232	4.8468	0.048468
150	2.67003	5.39997	0.0359998
200	2.4648	5.6052	0.028026
250	1.9261	6.1439	0.0245756
300	1.83874	6.23126	0.020770867
400	1.60735	6.46265	0.016156625
500	1.4588	6.6112	0.0132224
750	1.21452	6.85548	0.00914064
1000	1.09304	6.97696	0.00697696

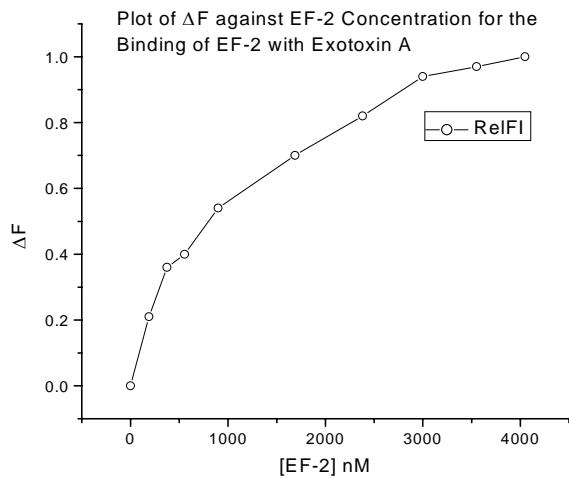


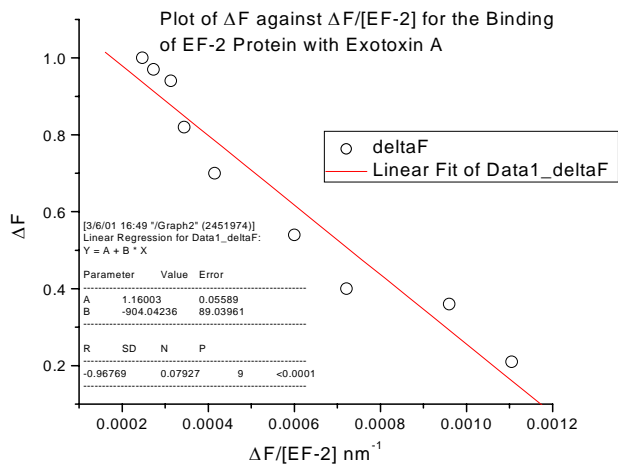


$$K_L = K_d = -(-46.2 \mu\text{M}) = 46.2 \mu\text{M}.$$

4.

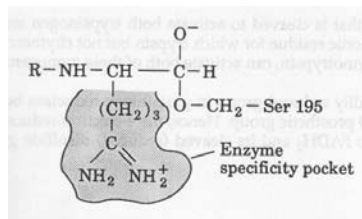
EF-2 (nM)	Relative FI	Delta F	Delta F/[EF-2] nM ⁻¹
0	0	0	--
190.1	0.21	0.21	0.0011
375	0.36	0.36	9.60E-04
554.8	0.4	0.4	7.21E-04
900	0.54	0.54	6.00E-04
1687.5	0.7	0.7	4.15E-04
2382.4	0.82	0.82	3.44E-04
3000	0.94	0.94	3.13E-04
3552.6	0.97	0.97	2.73E-04
4050	1	1	2.47E-04





$$K_L = K_d = -(-904.0 \text{ nM}) = 904 \text{ nM.}$$

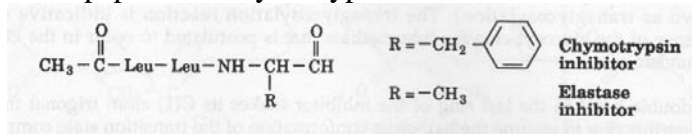
5. Benzamidine is a cationic and quite bulky molecule that binds in the specificity pocket of trypsin thereby blocking true substrates from binding to the enzyme. Leupeptin



resembles a polypeptide. Its cationic guanidino side chain binds in the specificity pocket of trypsin and its C-terminal aldehyde group occupies the position normally held by the carbonyl group of the scissile peptide. This aldehyde group reacts, as does the normal substrate, with Ser 195 to form a tetrahedral adduct. However, the catalytic reaction cannot

progress beyond this point since the resulting hemiacetal ion lacks the proper leaving group.

Such transition state analogues will inhibit chymotrypsin and elastase if they have the proper terminal side chain to bind in the specificity pocket of their respective enzymes. Chymotrypsin requires a bulky aromatic group to bind in its specificity pocket whereas elastase preferentially binds small nonpolar side chains such as CH_3 . Thus, examples of leupeptin-like chymotrypsin and elastase inhibitors are:



6. If the soybean trypsin inhibitor (a 21.5 kDa protein) was not removed from tofu, it would inhibit the trypsin in the intestine. At best, this would reduce the nutritional value of the meal by rendering the protein indigestible. It might very well also lead to intestinal upset.
7. Virtually all drugs used in the management of disease are competitive inhibitors of enzymatic reactions (transition state analogues in the best of cases). The HIV-1 protease inhibitors are synthetic transition state analogues of the regions of the HIV polyprotein cleaved by HIV-1 protease. These synthetic analogues bind tightly to the

substrate binding site of HIV-1 protease and therefore preclude the binding of the native substrate, HIV polyprotein. A characteristic of competitive inhibition is that it may be overcome by an increase in the $[S]/[I]$ ratio. Thus, competitive inhibition of cellular processes is temporary and will be relieved as the concentration of the native substrate increases. The fact that drug therapy is temporary has obvious advantages in some instances, e.g., in the administration of local anaesthetics by a dentist, or in the mistaken administration of a drug. The disadvantage is that all drugs are somewhat less efficient than they would be if they were not competitive inhibitors, for example, irreversible inhibitors would have a longer lasting effect on the system being inhibited.

8. Since captopril contains a free thiol, it is likely that the sulfhydryl group probably binds to the zinc ion at the active site, and its carboxylate group very likely occupies the recognition site for the C-terminus of the substrate.
9. Since His 134 is thought to stabilize the negative charge on the carbonyl oxygen in the transition state, the protonated imidazole ring (which carries a positive charge) must be the active species. That being the case, the enzyme velocity v_o should be half of the V_{max} at a pH of 6.5 (the pK of an unperturbed histidine side chain in a protein). Raising the pH above 6.5 will remove protons from the imidazole ring, thus causing a decrease in v_o ; lowering the pH below 6.5 will have the reverse effect.