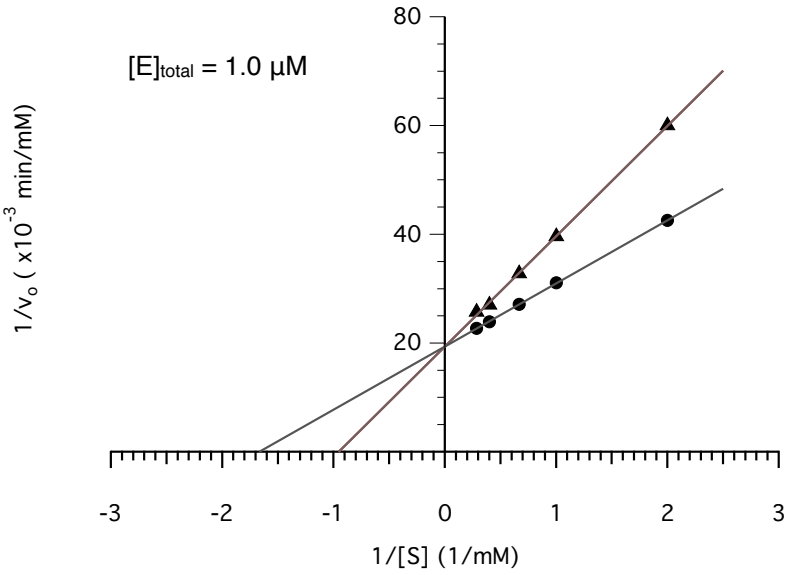
Chem 352, Fundamentals of Biochemistry
Lecture 4 – Supplemental Questions

1. Prostaglandins are members of a class of molecules called *eicosanoids* that are responsible for triggering inflammation along with the associated pain. Prostaglandins are derived from a 20-carbon fatty acid named *arachidonic acid*. A key enzyme in the synthesis of the prostaglandins is a cyclooxygenase called *COX-2*, which converts arachidonic acid to the prostaglandin intermediate PGG_2. Ibuprofen is a non-steroidal, anti-inflammatory drug (NSAID), which is able to reduce inflammation and pain by inhibiting the *COX-2* enzyme. Below are the results of a kinetics study of the *COX-2* enzyme in the presence and absence of ibuprofen. (v_o is the initial reaction rate and [S] is the arachidonic acid concentration.)



   a. Can you propose a location on the enzyme, relative to the substrate binding site, where the ibuprofen is most likely binding? Clearly state the evidence you have for your claim.

   b. If you were able to focus your attention on a single *COX-2* enzyme molecule, on average, how many reactions would it be carrying out per second when completely saturated with substrate? Clearly show your calculations.

   c. What effect would the presence of ibuprofen have on the observation you made in part b? Clearly state your reasoning.

   d. Is it possible to increase the catalytic efficiency of the *COX-2* enzyme? Clearly state the evidence you have for your claim

2. In class we discussed how the enzyme *hexokinase*, which catalyzes the reaction shown below, provides a good example of Daniel Koshland's "induced fit" model for substrate binding.

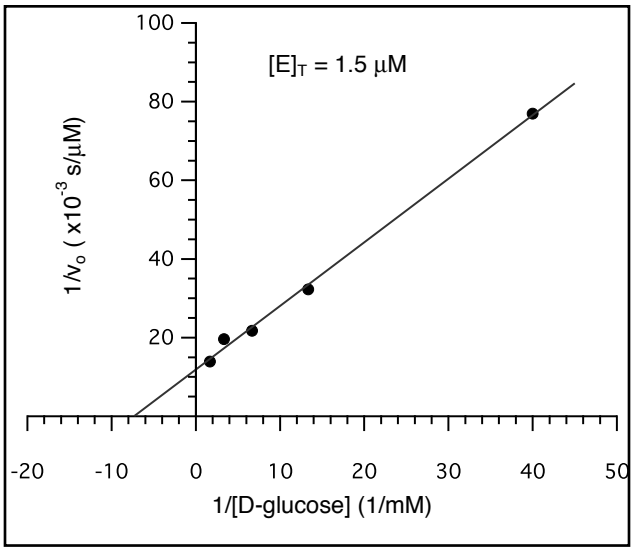
   a. Explain what the "induced-fit" model for substrate binding proposes.

b. What class of reaction does hexokinase carry out?_____

c. Using a Fischer projection, draw the chemical structure for D-glucose.

d. Using a Haworth projection, draw the chemical structure for β-D-glucose 6-phosphate in its pyranose ring form.

e. Based on the results of a kinetics experiment shown to the right that was collected using a hexokinase concentration of 1.5 μM, how many molecules of D-glucose does each hexokinase enzyme molecule convert to D-glucose 6-phosphate per second? Show your calculations and *circle* your answer. (v_o is the initial reaction rate for a given D-glucose concentration.)



f. Are the kinetics for the hexokinase catalyzed reaction limited by the rate that substrate molecules are able to diffuse in to the active site? Show your calculations and clearly state the evidence for your claim.

g. In a separate kinetics experiment, the presence of an *uncompetitive* inhibitor is found to reduce the V_{max} to 1/3 of its uninhibited value. On the Lineweaver-Burk plot shown on the previous page, sketch a line that represents the expected results for this experiment.

h. Calculate the predicted apparent K_M value for the hexokinase reaction in the presence the inhibitor for the experiment described in part g.

i. Does the presence the inhibitor for the experiment described in part g affect the apparent affinity of the hexokinase for D-glucose? If so, does it *increase* or *decrease* the affinity. Clearly state the evidence you have for your claim.

3. In class, we examined in detail the step-by-step mechanism for the reaction that is catalyzed by the serine protease *chymotrypsin.* We saw that it provides us with a number of examples of the different strategies used by enzymes to increase the rates for enzyme-catalyzed reactions.

a. For the players listed below from chymotrypsin, use one or two complete sentences to describe the contribution that each makes to implementing one of these strategies:

- Histidine-57:
- Serine-195
- Aspartate-102
- The oxyanion "hole"

b. Of all of the strategies that we discussed in class, which of these typically makes the greatest contribution to the increased rate relative to the uncatalyzed reaction? _____