Chem 135: Exam I

11 February 2010

Please provide all answers in the space provided. Extra paper is available if needed. You may not use calculators for this exam. Including the title page, there should be 12 single sided pages in this exam booklet.

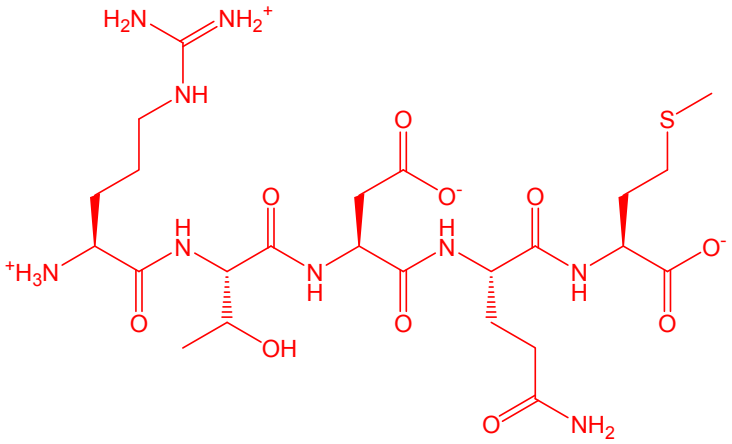
¡ Good luck !

Name: **KEY**

(1) _____ (7 points)
(2) _____ (18 points)
(3) _____ (20 points)
(4) _____ (10 points)
(5) _____ (10 points)
(6) _____ (18 points)
(7) _____ (17 points)

TOTAL _____ (100 points)

1. a. Draw the peptide: **RTDQM** at pH 7.0 (with the correct stereochemistry at ALL C_α-carbons).



b. What is the total charge of the peptide at pH 1? +2, N-terminus and R are positively charged, there are formal no negative charges

c. What is the total charge of the peptide at pH 14? -2, C-terminus and D are negatively charged, the are no formal positive charges

2. While sampling for unique microbial activity in the "fountain" in front of Lewis Hall, you find a new protein and take it back to the laboratory to study. Luckily this new protein is very small so you name it Tiny. The first thing you do is to analyze the primary structure of Tiny by reducing and cleaving it with different reagents in separate tubes. You are then able to separate and sequence the different peptides in each tube and find the following:

Tube 1: PGCGAKLISTCKFGNWEDG, M, EERHCVLRLWM
Tube 2: FGNWEDG, LWMPGCGAK, HCVLR, LISTCK, MEER
Tube 3: MEERHCVLRLW, MPGCGAKLISTCKF, GNW, EDG

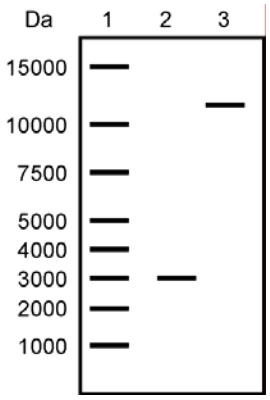
a. What cleaving agent did you use for each tube? Describe the specificity of each cleaving agent.

1. Cyanogen Bromide, cleaves after M
2. Trypsin, cleaves after R and K (positively charged residues)
3. Chymotrypsin, cleaves after F, W, and Y (aromatic residues)

b. What is the sequence of Tiny using single-letter codes for the amino acids?

MEERHCVLRLWMPGCGAKLISTCKFGNWEDG

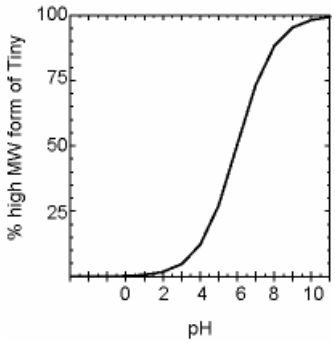
c. Using a technique called gel electrophoresis, you can determine the size of Tiny under reducing and non-reducing conditions. In the gel below, Lane 1 contains the molecular weight marker as a reference. Lane 2 contains Tiny under reducing conditions and Lane 3 contains Tiny under non-reducing conditions. Draw the reaction that occurs on Tiny when you add a reducing agent and describe the quaternary structure of Tiny.



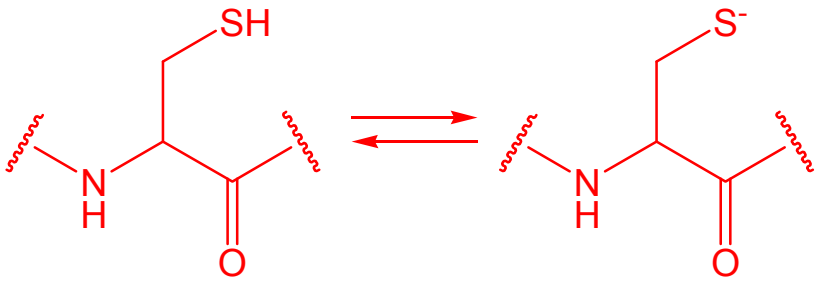
When a reducing agent is added the disulfide bond is broken.

Gel electrophoresis shows that Tiny is a homotetramer. Under reducing conditions, when the monomers cannot form quaternary structure the size is 3000 Da while under non-reducing conditions when quaternary structure forms the size is 12000 Da (four times larger).

d. After Tiny has been reduced, you monitor the pH dependence of the formation of the high-molecular weight band seen in Lane 3 of the gel from 2c. Please explain briefly showing a chemical equation for the protonation/deprotonation event and explain if the equilibrium is shifted. If there is a shift, please give a possible explanation for the observed pK_a.



In order to form the disulfide bond the Cys must become deprotonated, see chemical equation below.

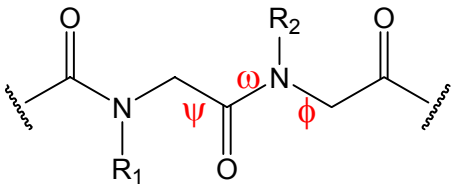


The pKa is shifted. Normally, the pKa of Cys is 8.2 in the plot the pKa is ~6 (the pH at which 50% of the Cys are deprotonated and can be used to form the high-molecular weight band). Since the pKa is lowered, the deprotonated form of Cys is favored, perhaps because of a positively charged residue (R or K) near Cys.

e. You decide to use a technique called site-directed mutagenesis, where you mutate a particular amino acid to another one and study its effect on the protein behavior. If you chose to make a conservative substitution (i.e. the closest structurally-related amino acid that cannot carry out the reaction you propose in part 2d), which amino acid would you choose and why?

I would replace it with Ser. The only difference by mutating the residue to S would be to replace the sulfur with oxygen, which would no longer be able to form disulfide bonds but is structurally the most similar.

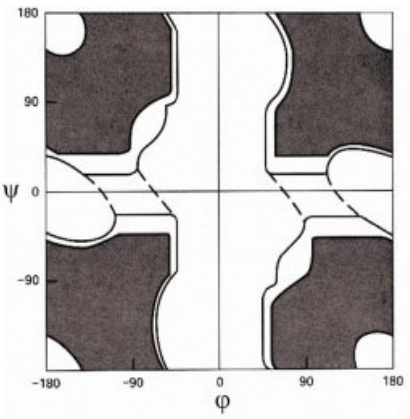
3. Some researchers are interested in studying peptide and protein mimics called peptoids. Peptoids differ from peptides by the fact that their side-chains are attached to the backbone via the amide nitrogen rather than C_α.

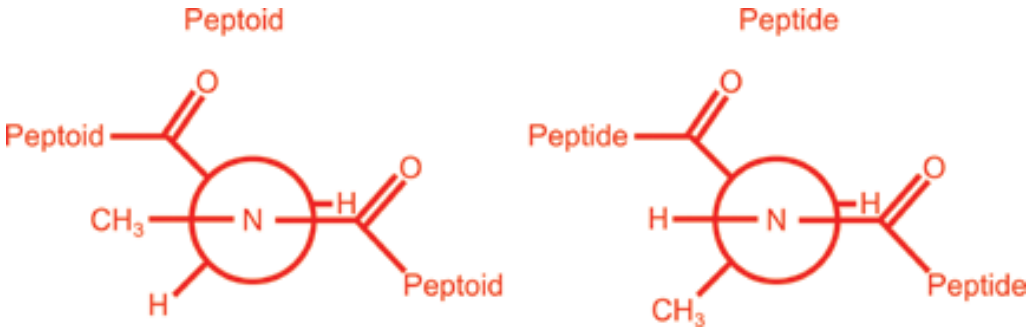


a. Do you expect peptoids to have the same hydrogen-bonding capabilities as a peptide of the same sequence (same R group)? Briefly explain.
The R groups would show the same hydrogen-bonding capabilities, but the backbone could not. The backbone still contains hydrogen bond acceptors (the carbonyl). There are no longer hydrogen bond donors in the backbone, because when the R group is bound to the backbone nitrogen there is no longer a hydrogen bound.

b. Peptoids are also observed to be more flexible than the corresponding peptide. For example, the cis and trans amide bond geometries are approximately isoenergetic unlike peptides in which the trans isomer is highly favored. Mark the bond rotation on the peptoid figure above that corresponds to the amide bond (ω) and briefly explain this observation.
In the peptoid, the Cα has no R group so there is less steric bulk to clash when both of the carbons are on the same side of the peptide bond. At the same time, there is also now an R group attached to the nitrogen in place of a hydrogen atom in a peptide.

c. Although peptoids are more flexible, they can also adopt stable three-dimensional structures that have been structurally characterized by X-ray crystallography. In addition, researchers have been interested in determining a Ramachandran-type plot for peptoids to elucidate the allowed values of ϕ and ψ. A Ramachandran plot for a peptoid where R = $-CH_3$ is shown below. On the figure above, mark the rotations corresponding to ϕ and ψ and explain the Ramachandran plot using Newman projection for ϕ and how it differs from a typical amino acid.



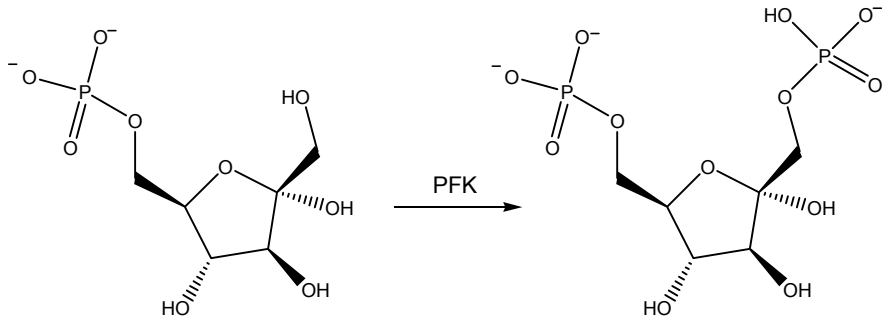


Both pictures above are drawn at -120°.
The φ angle is as likely to be around 120° as it is -120° in the peptoid. This is the result of no R-group on Cα to clash with the chain coming off of the nitrogen in the peptoid. In the peptide the methyl group would be eclipsed with the chain coming off the nitrogen at 120°.

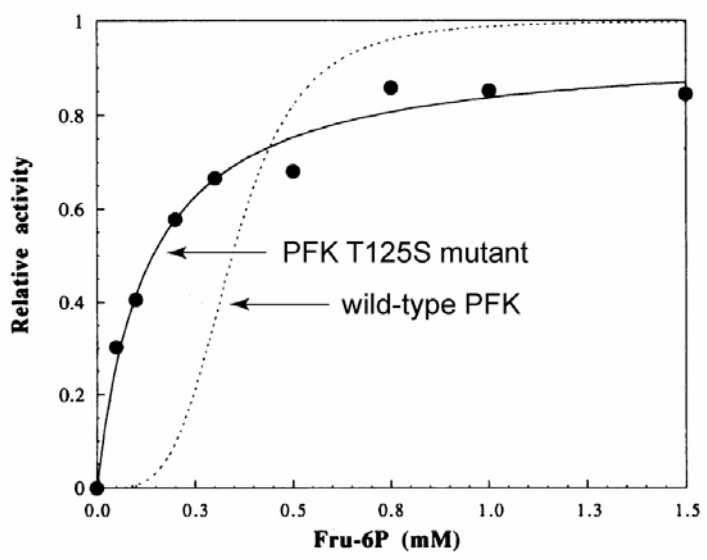
The Ramachandran plot for the peptoid is also semetric when reflected across both axes because the backbone is not chiral (unlike the peptide plot).

d. Which amino acid do you think would be most comparable to the peptoid from part 3c and briefly explain.
Glycine would be most comparable to the peptoid from 3c. In both cases the Cα has the same atoms bound. The only difference between them is that the peptoid has the methyl group on the backbone nitrogen. So you would expect the Ramachandran plots to be pretty similar.

4. The enzyme phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate (Fru-6P) to form fructose-1,6-bisphosphate (Fru-BP) using adenosine triphosphate (ATP).

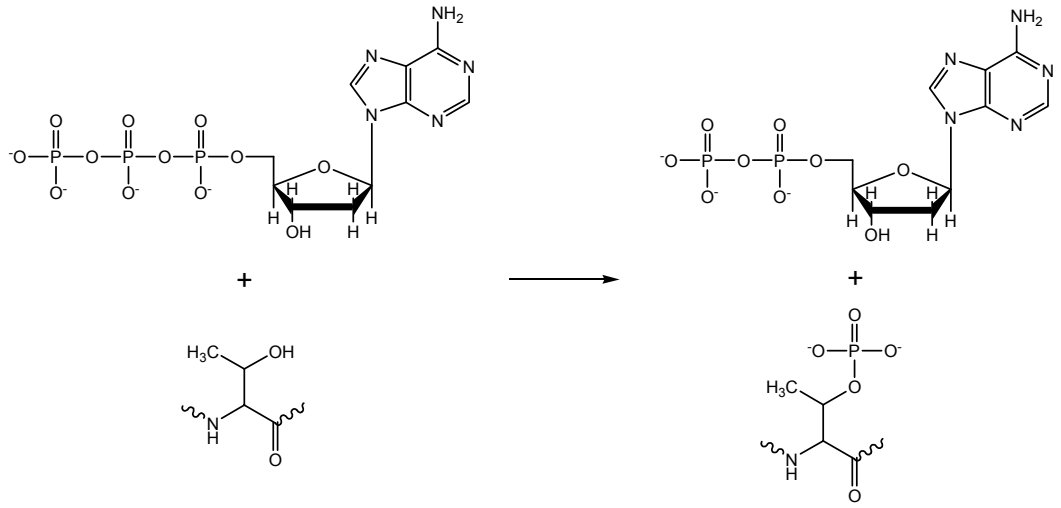


a. The graph below shows a saturation curve of wild-type PFK with respect to the substrate Fru-6P. What sort of behavior does PFK exhibit?



Cooperative (allosteric)

b. Further studies of PFK show that there is an intermediate step in the catalytic cycle where T125 is phosphorylated. If T125 is mutated to serine (PFK T125S mutant), the enzyme is still capable of catalyzing the reaction, but the shape of the curve changes to that observed in the graph shown in part 5a. Please briefly describe what has happened in the mutant to account for the changed saturation behavior in terms of substrate binding and K_D.



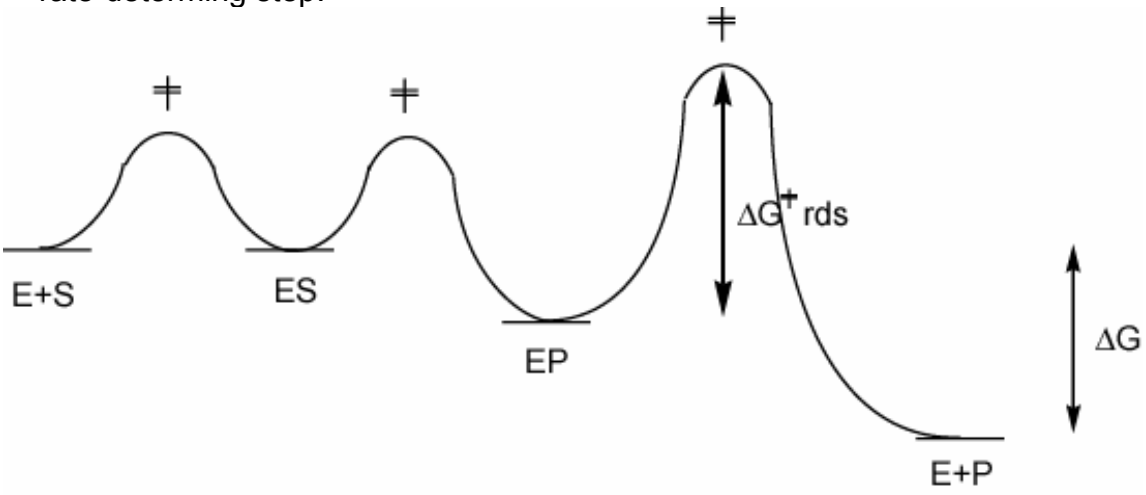
The phosphorylation of the threonine in the native PFK leads to a change in the protein's structure that allows it to then be more active. The T125S mutant does not show the same allosteric behavior and instead shows a decreased Km (~kd) and increased ability to bind substrate.

c. Using the graph from part 5a, estimate the difference in relative activity between wild-type PFK and the T125S mutant at 0.3 mM Fru-6P. Please describe one possible outcome of this difference in relative activity if this mutation were to occur in living cells.

The difference in activity is ~ 40%. If this were to occur in living cells, the mutant enzyme would be significantly more active at a lower concentration of substrate possibly running the cell out of substrate and ATP.

5. Enzymes are often inhibited by the product of their reaction, a phenomenon that is creatively termed "product inhibition" as a stable EP complex is formed.

a. Draw an energy diagram for an enzyme-catalyzed reaction that shows the ES and EP complexes as discrete intermediates for the case where product inhibition occurs and product release is rate-limiting. Please label the activation energies and indicate the activation energy that corresponds to the rate-determing step.

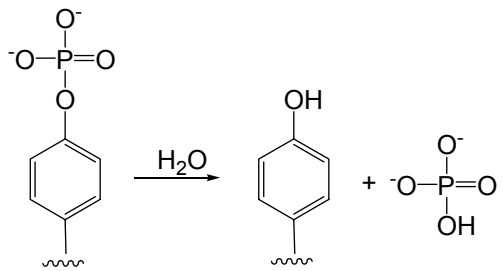


c. Indicate on the graph using a vertical arrow, the overall ΔG for the reaction.

d. What is the equilibrium constant for this reaction?

$K_{eq} = [P]/[S]$

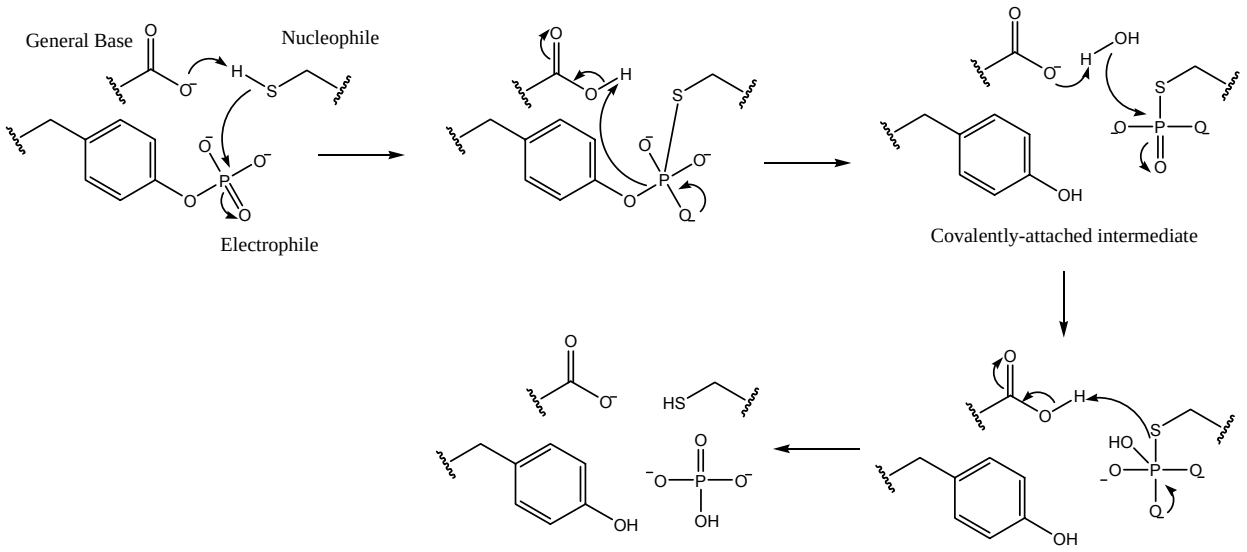
6. Protein phosphorylation is a key modification that can regulate their activity since it can change interactions between proteins, introduce conformational change, or change the localization of a protein. Protein kinases are enzymes that introduce phosphoryl groups onto proteins, a reaction that can then be reversed by the action of protein phosphatases that remove the phosphate group by hydrolysis. You have been studying a pathway and have found a key regulatory phosphatase that you name Sprouty because mutations in the Sprouty gene cause the formation of extra toes in rats. Luckily, you are able to quickly obtain a crystal structure and see the active site of Sprouty, which will really help you out in your biochemical experiments.



a. In the active site of Sprouty, you observe many different polar residues that could be involved in catalysis. You mutate all of these residues to alanine and measure their k_{cat} and K_m values. Looking at the table of mutations below, fill in the predicted function of the residue in terms of catalytic, substrate recognition, or none.

Sprouty	k_{cat} (s^{-1})	K_m (μM)	Predicted function of residue?
Wild-type	100	100	
H65A	95	1000	Substrate recognition
C89A	<0.1	152	Catalytic
D101A	1	125	Catalytic
S130A	65	103	Catalytic or None
D127A	104	110	None
E142A	98	1500	Substrate recognition

b. Using a combination of a ^{32}P-radiolabelled phosphotyrosine-containing peptide substrate and mass spectrometry, you determine that Sprouty goes through an intermediate where the phosphate group is covalently attached. Draw a detailed arrow-pushing mechanism for Sprouty for the phosphotyrosine hydrolysis reaction above. Label the nucleophile, electrophile, as well as general/specific acids and bases.



c. Although the C89A Sprouty mutant does not show detectable catalytic activity, you measure the k_{cat} of the C89S mutant to be 5 s^{-1}. Looking at the crystal structure, you design a second-site mutation at residue 130 that is adjacent to residue 89 to make the double-mutant C89S S130H. The double-mutant has a k_{cat} of 55 s^{-1}. Please give a possible explanation for these observations based on what we have discussed in class.

The D101 has too low of a pKa to sufficiently deprotonate the serine in the C89S to then act as a nucleophile yielding a low kcat. For the native protein, based on the slight catalytic activity S130 appears to play, it may play a role in stabilizing the negative charge that forms on the cysteine through H-bonding, lowering the pKa of cysteine so that it is more easily deprotonated. When S130 is mutated to a His, the His likely plays the same role, lowering the pKa of the C89S, such that it can be deprotonated more easily and act as a nucleophile.

7. Isozymes are enzymes that catalyze the same reaction but may have different biochemical and kinetic properties. You have isolated two isozymes of a glucose oxidase, one is from the intestine (IGO) and the other is from the liver (LGO), and are now comparing the kinetics of the IGO and LGO as part of your research project.

a. During your experiments, you show that IGO and LGO both obey Michaelis-Menten kinetics and measure their k_{cat} and K_m values. Which enzyme has a higher catalytic efficiency and explain your answer.

Isozyme	k_{cat} (min^{-1})	K_m (mM)
IGO	1×10^6	1
LGO	1×10^5	0.01

Catalytic efficiency is defined by kcat/Km

kcat/Km [IGO] = 10^6 $min^{-1}mM^{-1}$ kcat/Km [LGO] = 10^7 $min^{-1}mM^{-1}$

LGO has the higher catalytic efficiency

b. If the [glucose] = 0.1 M in the intestine, which is the faster enzyme in the intestine? For the faster enzyme, provide a simplified rate law and a value for the effective rate constant.

[Glucose] >> Km, so the system is saturated

$$V_o = \frac{k_{cat}[S][E_T]}{K_m + [S]} = k_{cat}[E_T] \qquad k_{cat} = V_o [E_T]^{-1} = 10^6 \text{ min}^{-1}$$

($K_m + [S] \sim 0$)

IGO is the faster enzyme

c. If the [glucose] = 10^{-6} M in the liver, which is the faster enzyme in the intestine? For the faster enzyme, provide a simplified rate law and a value for the effective rate constant. [Glucose] << Km

$$V_o = \frac{k_{cat}[S][E_T]}{K_m + [S]} = \frac{k_{cat}[E_T][S]}{K_m} \qquad k = \frac{k_{cat}}{K_m} = 10^7\ mM^{-1}\ min^{-1}$$

($[S]$ in the denominator ~ 0)

LGO is the faster enzyme

d. If the enzyme from the intestine (IGO) is transferred to the kidney, where the [glucose] = 100 mM, would the overall turnover of glucose be faster if k_{cat} were increased 10-fold or K_m were decreased 10-fold? Please briefly explain your answer.

Because [Glucose] > Km, the system is saturated, so the rate law becomes

$$V_{max} = k_{cat}[E_T]$$

and only relies on Kcat. Thus, **increasing the kcat 10-fold** would increase the overall turnover more than decreasing the Km