Name: Signature: DocID:

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Quantitative Physiology I / Molecular and Cellular Systems BMEN E4001x / FA25
Midterm Exam 1

Exam instructions:

- Please report your answers on these pages.
- Do not use the backside of the pages for answers.
- A blank page is included after each long-response section. Please use for any additional work you wish on that problem.
- Draw a box around your final answers.
- For restroom breaks, only one student per room at any one time.

Statement of individual work:

By placing your name, UNI, and signature at the top of this page, you attest that this work represents your individual efforts, in compliance with the academic honesty guidelines established for this university and this exam.

Some Useful Constants:

N_A = Avogadro's Number = 6.02×10^{23} molecules/mole
σ_{O2} = solubility of oxygen in aqueous solution = 1.4×10^{-6} M/mmHg
k_B = 1.38×10^{-23} J/K

Unit conversions

$1\ m^3 = 1000\ L$
$1\ J = 1\ kg*m^2/s^2$

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Part 1) Multiple Choice / True False (25 points total)
5 points for correct answer, 0 for wrong, 2 for no answer,
5 automatic points for being awesome.

___A__ (**A** or **B**) You have two inhibitors to an enzyme, E. Inhibitor A works through a non-competitive scheme while B works through a competitive scheme. If you seek to minimize the activity of E in a patient, which inhibitor would you use? These inhibitors would be used at a concentration of 10 mM, and the K_i for both enzymes is 5 mM. Assume a substrate concentration of 1 mM, and K_m = 0.1 mM.

Comparison of inhibited enzyme behaviors. Computation of two equations, or recognizing that since [S] >> K_m, rates are saturated. Thus, the non-competitive inhibitor will usually have a bigger effect.

___F__ (**T**)rue or (**F**)alse: The Hill equation provides a mechanistic explanation how the sites in hemoglobin coordinate to make the curve of saturation vs. O_2 concentration deviate from Michaelis-Menten behavior.

The mechanism for cooperative binding of oxygen by hemoglobin is conformational change of the protein. The Hill equation captures the behavior of this interaction, but not the mechanism.

___B__ (**A** or **B**) The binding of multiple oxygen molecules to Hemoglobin is an example of (**A**) multivalent binding or (**B**) cooperative binding.

___T__ (**T**)rue or (**F**)alse: Increasing the partition coefficient of a molecule within a material increases transport across that material in response to a given concentration gradient.

$$J=1/R*\Delta C$$

$$R = L/(D*A*\beta).$$

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Part 2) Written Response (75 points total)

2) Enzyme Kinetics (30 points)

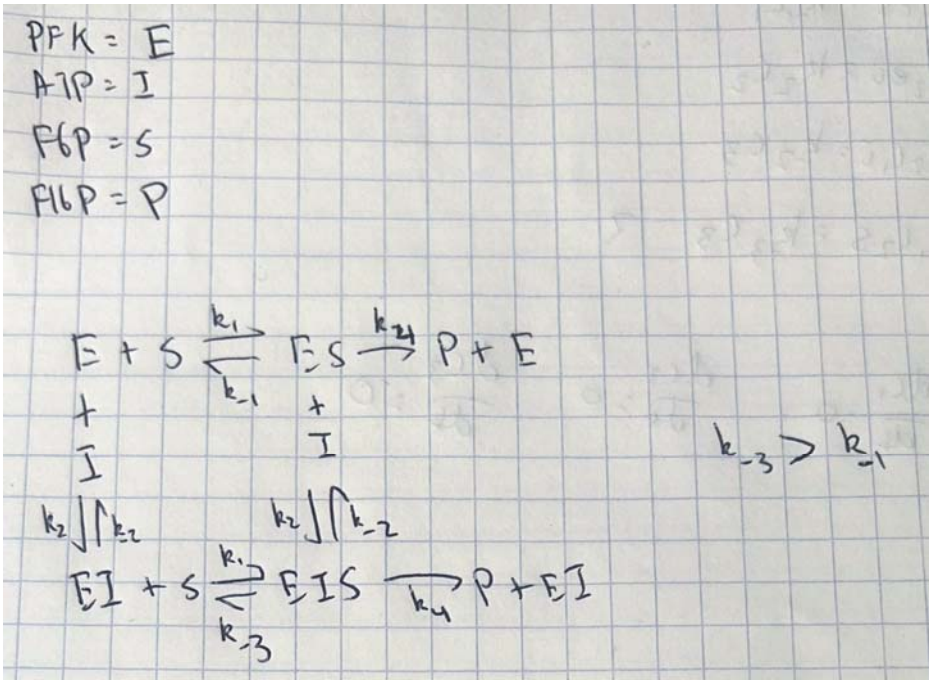
Regulation of certain isoforms of phosphofructokine (PFK) by ATP happens by allosteric inhibition, in which binding of ATP decreases affinity of PFK for its substrate fructose-6-P (F6P).

- PFK acts enzymatically on F6P to generate fructose-1,6-P (F16P), regenerating PFK.
- ATP can bind to an inhibitory site on PFK that is separate from the F6P catalytic site.
    - F6P binding to PFK (forward reaction rate) is not affected by bound ATP.
    - Dissociation of bound F6P from PFK (reverse rate), is increased by bound ATP.
    - Binding of ATP to PFK is independent of the presence of F6P at the catalytic site.
- The rate of conversion of F6P bound to the catalytic site of PFK into F16P is independent of the presence of ATP at the inhibitory site.
- Thus, PFK is the enzyme (E), acting on substrate (S) F6P to produce product (P) F16P. This reaction is modulated by ATP acting as an inhibitor (I)

The goal of this problem is to determine reaction rate as a function of E, S, and I concentrations.

This question is a variation on HW1Q1, Enzyme kinetic

2.1) Draw a diagram of this reaction pathway. Include fundamental rate constants. Indicate which rate constants must be equal from the problem statement; one way is to use the same rate constant label in the corresponding steps, as was done in our discussion of non-competitive inhibition (10 pts)



Variations on this layout, like the parallel reactions shown in class, are also good.

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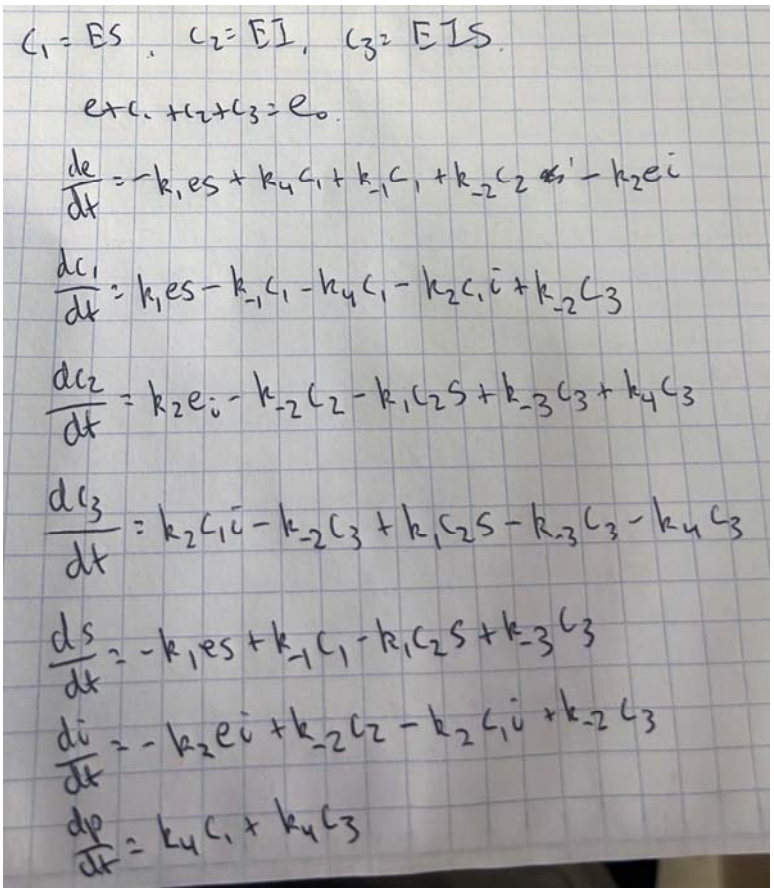
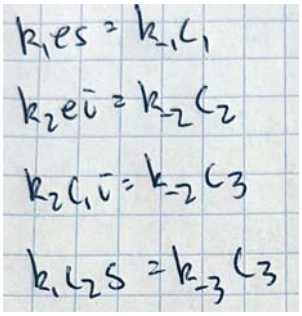
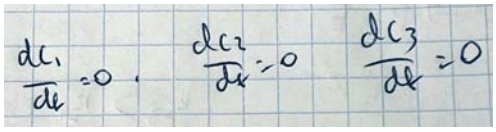
2.2) List the differential equations for E, S, I, intermediate complexes, and P describing system kinetics. Also provide a conservation equation for different forms of PFK. (10 pts)



2.3) List the equations that result from applying the Equilibrium approximation. (5 pts)



2.4) List the equations that result from applying the Quasi-steady state approximation. (5 pts)



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3) Sedimentation (20 points)

Protein aggregation is a problem in biomolecular assays. As such, removal of aggregates is an essential step of many experimental protocols. In this problem, you have a sample of a protein termed A in a 1-cm tall sample tube. Your goal is to remove aggregates from this sample using a centrifuge, which creates accelerations expressed as multiple of Earth's gravitational field, g. It is also refrigerated (4°C) to reduce protein damage. Very similar to HW2 Question 3:

- A is present in the tube in two forms – individual spherical proteins measuring 1.8 nm in diameter (volume = 3.0×10^{-21} cm^3) and spherical aggregates of 50 nm diameter (volume = 6.5×10^{-17} cm^3, note the change in aggregate size).
- The density of A, in either form, is 1.3 g/ml.
- The reaction is carried out in a buffer of density = ρ = 1 g/ml and viscosity = η_W = 1×10^{-2} poise (both at 4° C); 1 poise = 1 dyne*s/cm^2 = 1 g/(s*cm)

Some constants for this problem:

$k_B = 1.38 \times 10^{-23}$ J/K

T = 277 K (4°C), refrigeration

Earth's gravitational field = g = 9.8 m/s^2

k_BT (at 277 K) = 3.82×10^{-21} J = 3.82×10^{-18} $g*m^2/s^2$

This question is a variation on HW2Q3, sedimentation / buoyancy of milk components

3.1) What is the minimum acceleration (for example, 1,000 X g) needed to separate out the aggregates in 1 hr? Use the criteria of HW2 Question 3, and show your work. (10 pts)

Velocity = $m_{net}*a/(6*\pi*\eta_w*R)$; a = $(6*\pi*\eta_w*R)*Velocity/m_{net}$

m_{net}= 1.95E-17 g, R=25 nm, nu= 1E-2 g/(s*cm) = 1 g/(s*m)

needed velocity is 0.01 m / 3600 sec = 2.78E-6 m/s

a = $(6*\pi*1(g/(s*m))*2.5E\text{-}8m * 2.78E\text{-}6(m/s)/1.95e\text{-}17g = 6.72E4$ m/s^2 = 6855 * g

3.2) Your centrifuge is capable of very high accelerations. What is the maximum acceleration that can be used while avoiding separation of individual proteins from solution? Assume centrifugation is carried out to equilibrium/steady state. Use the criteria of HW2 Question 3 and show your work. (10 pts)

$z^* = k_BT/(m_{net}*a)$; a= $(k_BT/m_{net}) / z^*$

$m_{net} = 9 \times 10^{-22}$ g;

for z* = 0.01 m, a = $3.82E\text{-}18(g*m^2*s^{-2})/(9E\text{-}22g))/1E\text{-}2m$= 4.24E5 m/s^2 = 4.3E4 X g

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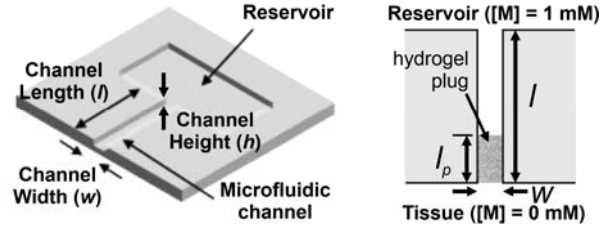
4) Diffusive transport (25 points)

Your lab is designing an implantable microfluidic system for controlled delivery of a drug M to tissues. This consist of a well-stirred reservoir ([M] = 1 mM) connected to tissues by a microfluidic channel, measuring 20 μm wide (*w*), 20 μm high (*h*), and 1 mm in length (*l*).



adapted from doi:10.106/actbio.2017.12.013

In the initial design, this is filled with media. However, cells from the tissue are able to crawl into and block the microchannel. To address this, the end of the microchannel closest to the tissue is filled with a hydrogel plug (right side of the figure). This plus makes up the last 200 μm of the channel length, l_p. The properties of M in the various regions are given below.

Layer	*[M]*	*D (μm^2/sec)*	*β*
Reservoir	1 mM (well-stirred, uniform concentration)	1000	1
Media		1000	1
Hydrogel		500	.8
Tissue	0 mM (well-stirred, uniform concentration)	1000	1

This question is a variation on HW2Q2, diffusion based transport through engineered systems
In this case, transport is along the channel, but the question is still based on diffusive barriers in series with each other.

4a) What is the total flux of M into tissues in the initial (media only) design? (10 points)

Total flux, $J = (DA/L)*\Delta C$

$A = w*h = 400\ \mu m^2 = 4E\text{-}10\ m^2$

$L = 1E\text{-}3\ m$

$D = 1E\text{-}9\ m^2/s$

$\Delta C = 1\ mM - 0\ mM = 1\ mM = 1\ mol/m^3$

$J = 4E\text{-}16\ mol/s$

4b) What is the total flux of M into tissues in the modified (plug) design? (10 points)

Total flux, $J = (1/R_T)*\Delta C$; $R_T = R_M+R_H = L_M/(A_M D_M \beta_M)+ L_H/(A_H D_H \beta_H)$

$A_M = A_H = 4E\text{-}10\ m^2$; $L_M = 8E\text{-}4\ m$; $L_H = 2E\text{-}4\ m$; $\Delta C = 1\ mol/m^3$;
$D_M = 1E\text{-}9\ m^2/s$; $D_H = 5E\text{-}10\ m^2/s$; $\beta_M = 1$; $\beta_H J = 3.08E\text{-}16\ mol/s$

4c) The length of the channel can be changed. Maintaining a plug length, l_p, of 200 μm, what should the total channel length be to restore the original flux? (5 points)

Total flux, $J = (1/R_T)*\Delta C$; $R_T = R_M+R_H = L_M/(A_M D_M \beta_M)+ L_H/(A_H D_H \beta_H)$

Want $J = 4E\text{-}16\ mol/s$, Solve for L_M with

$A_M = A_H = 4E\text{-}10\ m^2$; $L_H = 2E\text{-}4\ m$; $\Delta C = 1\ mol/m^3$;
$D_M = 1E\text{-}9\ m^2/s$; $D_H = 5E\text{-}10\ m^2/s$; $\beta_M = 1$; $\beta_H L_M = 500\ \mu m$, Total length = $L_M + L_H = 700\ \mu m$.