20.320 Exam 2
Thursday November 17th
9:35-10:55

Instructions:

0. Write your name of the front cover of the blue book.

1. Answer all questions in the blue books. This exam paper will not be graded.

2. All questions can be answered in at most a few sentences. We will deduct points for excessively long replies, even if they contain the right answer.

3. State all assumptions for each problem.

4. In order to pace yourself please note that the maximum possible score on this exam is 100 – note that there are 4 total questions.

Question 1. (18 points total)

Binding of SHC to EGFR has been implicated in activating the ERK MAPK cascade. To block this interaction a large-scale screen of a small molecule library has been performed. This analysis identified a single strong binder (MITSC1, molecular weight: 615 g/mol) to the SHC SH2 domain. Obviously, further biophysical characterization is warranted. Specifically, kinetic parameters will help to determine how long the compound will stay bound to the SH2 domain (molecular weight of the SHC SH2 domain is 10,520 Da (note 1Da = 1g/mol)), which will inform as to the potential for this compound to be used as a therapeutic.

a) (5 points) To generate these kinetic parameters an SPR experiment is performed, but there is only a limited amount of SHC SH2 domain available, such that only two analyses were performed. Calculate K_d, k_{on}, and k_{off} given that t_{obs} = 14 minutes with a protein concentration of 10^{-8} M, and t_{obs} = 27 minutes with an initial protein concentration of 10^{-9} M

$$t_{obs} = \frac{1}{k_{obs}} = \frac{1}{k_{on}[L_0] + k_{off}}$$

$$27\,min = \frac{1}{k_{on}10^{-9} + k_{off}}$$

$$14\,min = \frac{1}{k_{on}10^{-8} + k_{off}}$$

Rearranging,

$$k_{on}10^{-9} + k_{off} = \frac{1}{27\,min}$$

$$k_{on}10^{-8} + k_{off} = \frac{1}{14\,min}$$

Subtract the two equations to get

$$k_{on}(10^{-8} - 10^{-9}) = \left(\frac{1}{14} - \frac{1}{27}\right) min^{-1}$$

Solving, we get k_{on} = 3.821e6 M^{-1} min^{-1} (or 6.36e4 M^{-1} s^{-1}). If we then plug that into either equation, we get k_{off}=0.03322 min^{-1} (or 5.5e-4 s^{-1}). $K_D = k_{off} / k_{on}$ = 8.692e-9 M.

b) (5 points) Given the limited amount of SHC SH2 domain available, you want the experiment to be as short as possible. With that said, it is still a good idea to have the system reach equilibrium prior to stopping the flow of the SHC SH2 domain. How long will it take to reach 95% equilibrium in each of the above cases?

$RU(t) = RU_{eq}(1-e^{-kobs * t})$

$$\frac{RU(t)}{RU_{eq}} = 0.95 = 1 - \exp(-k_{obs}t)$$

$$0.05 = \exp(-k_{obs}t)$$

$$\ln(0.05) = -k_{obs}t$$

$$t = \frac{\ln(0.05)}{-k_{obs}} = -t_{obs}(\ln 0.05)$$

= 41.9 minutes, 80.9 minutes for 10^{-8}, 10^{-9} M, respectively.

C) (4 points) For the above case of MITSC1 interacting with the SHC SH2 domain, does it make sense to perform an ITC experiment? Explain your answer.

There are a number of good reasons why we might not be interested in doing an ITC experiment. ITC does not yield kinetic parameters, and hence is of limited use if we are primarily interested in kinetics (which is the stated in the question).Additionally, it is stated we have limited material, and ITC requires a large amount of material (generally). Finally, the K_D is a bit low for ITC, technically outside the 10^{-8} to 10^{-5} M range.

D) (4 points) Regardless of your answer to (C), how much material, for both MITSC1 and the SHC SH2 domain, would you need to conduct an ITC experiment?

For ITC, we want a protein concentration in the range $10 < \frac{P_0}{K_D} < 100$, so say $P_0 = 20K_D$ = 1.7e-7 M. $[L]_0$ (concentration in injection syringe) should be about 10 times $[P]_0$, so say 1.7e-6 M. Since we need ~1mL protein solution and say we do 20 10uL injections, we need 1.7e-10 mol and 3.4e-10 mol of protein and ligand, respectively (arguably you might need to double the ligand amount to account for the injections into the reference cell. You can calculate these amounts using the known atomic masses.

Question 2. (36 points total)

As we have discussed in class multiple times, Epidermal Growth Factor Receptor (EGFR) signaling is a critical aspect of normal physiology, yet dysregulated EGFR signaling has been implicated in a variety of disease states, including multiple human cancers. Last year the Lemmon lab published a manuscript which claimed that EGFR dimerizes and then sequentially binds two molecules of EGF to finally form the signaling competent tetramer (comprised of two EGF and two EGFR molecules). This tetramer finally leads to phosphorylation and activation of many downstream pathways, including the ERK-MAPK cascade. To simplify this system, assume that the tetramer directly phosphorylates and activates ERK (you can assume a single activating phosphorylation site on ERK to simplify the model further). ERK phosphorylation is regulated by a constitutively active phosphatase (P'ase1).

A) (4 points) Create a schematic diagram of this system. For your model, you can assume that both EGFR proteins are identical.

B) (10 points) Write out the ordinary differential equations describing this system.

C) (4 points) In the manuscript, the authors claim that binding of the first ligand partially obscures the binding pocket for the second ligand, such that EGFR displays negative cooperativity in ligand binding. How does this affect your system of equations?

D) (6 points) Several labs have developed antibodies that block EGFR dimerization. Add this component to your system, assuming reversible antibody binding to the receptor, and write out any new equations. Sketch a plot estimating the level of ERK phosphorylation **versus amount of EGF added**. How does this diagram change with addition of different amounts of the dimerization blocking anti-EGFR antibody?

E) (6 points) In multiple publications it is claimed that there is a negative feedback loop in which ERK phosphorylates EGFR (the signaling competent tetramer) to shut down signaling. Add this loop to your system and write out any new equations (you do not have to add it to part A, but can if you

want a visual reference). Sketch a plot describing the ERK phosphorylation **over time following addition of EGF**, with and without this negative feedback loop.

F) (6 points) Given your natural scientific skepticism, you decide to use your model to test whether this same feedback loop (ERK phosphorylation of EGFR) could be a positive feedback instead of a negative feedback. Write out any new equations that would be necessary to test this theory. Sketch a plot describing the ERK phosphorylation **over time following addition of EGF**, with this positive feedback loop in place.

EGFR = Y Ligand = •

2. Bound EGFR = Ψ

Y = M YY = D ΨΨ = T

• = L ΨΨ = T·R

a)

(M)

$$Y + Y \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \Psi\Psi$$

$$\Psi\Psi + \bullet \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \Psi\Psi$$

$$\Psi\Psi + \bullet \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \Psi\Psi$$

$$\Psi\Psi + ERK \underset{k_8}{\overset{k_7}{\rightleftharpoons}} \Psi\Psi : ERK \xrightarrow{k_9} \Psi\Psi + Erk\text{-}P$$

$$ERK\text{-}P + P'ase \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} Erk\text{-}P : P'ase \xrightarrow{k_{12}} Erk + P'ase$$

b)

$$\frac{d[M]}{dt} = -k_1[M]^2 + 2k_2[D]$$

$$\frac{d[D]}{dt} = -k_3[D][L] + k_4[TR] - k_2[D] + k_1[M]^2$$

$$\frac{d[TR]}{dt} = k_3[D][L] - k_4[TR] - k_5[TR][L] + k_6[T]$$

$$\frac{d[T]}{dt} = k_5[TR][L] - k_6[T] - k_7[T][ERK] + k_8[T:ERK] + k_9[T:ERK]$$

$$\frac{d[ERK]}{dt} = -k_7[T][ERK] + k_8[T:ERK] + k_9[T:ERK] + k_{12}[Erk\text{-}P][P'ase]$$

$$\frac{d[ERK\text{-}P]}{dt} = +k_9[T:ERK] - k_{10}[ERK\text{-}P][P'ase] + k_{11}[Erk\text{-}P:P'ase]$$

c) Negative cooperativity causes k_5 to be less than k_3 (binding of the second ligand slower than binding of the first)

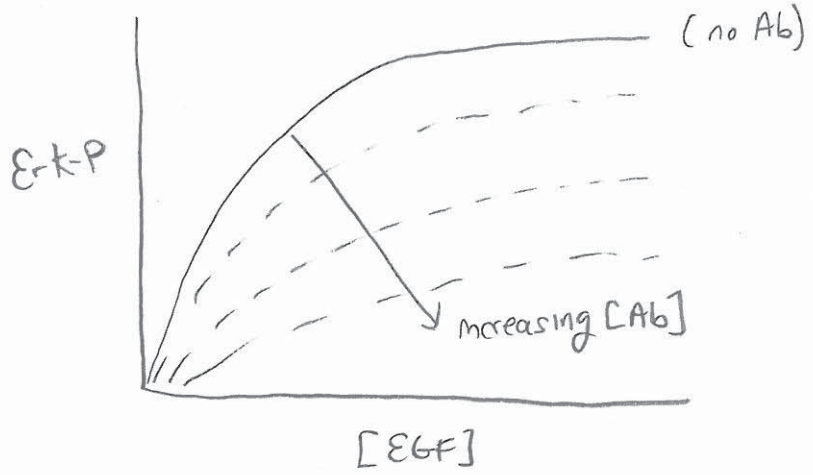
D)

D) Adding Ab to the system:

$$Y + Ab \underset{k_{14}}{\overset{k_{13}}{\rightleftharpoons}} Y:Ab$$

$$\therefore \frac{d[M]}{dt} = -2k_1[M] + k_2[D]^2 - k_{13}[M][Ab] + k_{14}[M:Ab]$$

$$\frac{d[M:Ab]}{dt} = k_{13}[M][Ab] - k_{14}[M:Ab]$$



E) Erk-P phosphorylates T in negative feedback.

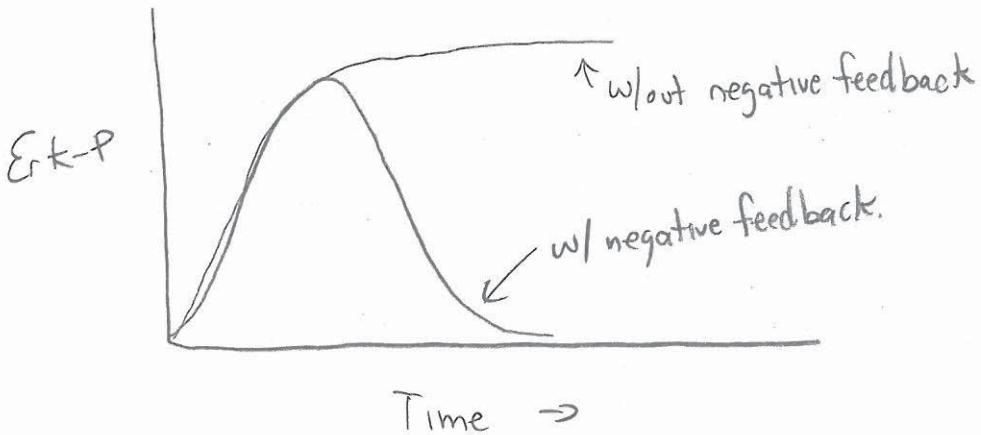
$$Erk\text{-}P + T \underset{k_{16}}{\overset{k_{15}}{\rightleftharpoons}} Erk\text{-}P:T \xrightarrow{k_{17}} Erk\text{-}P + T^*$$

(note: T^* is inactive tetramer)

Now:

$$\frac{d[Erk\text{-}P]}{dt} = k_9[T:ERK] - k_{10}[Erk\text{-}P][P'ase] + k_{11}[Erk\text{-}P:P'ase] + k_{16}[Erk\text{-}P][T] - k_{15}[Erk\text{-}P][T] + k_{17}[Erk\text{-}P:T]$$

$$\frac{d[T]}{dt} = k_5[TR][L] - k_6[T] - k_7[T][ERK] + k_8[T:ERK] + k_9[T:Erk] - k_{15}[Erk\text{-}P][T] + k_{16}[Erk\text{-}P:T]$$



F) Positive feedback from Erk-P to T

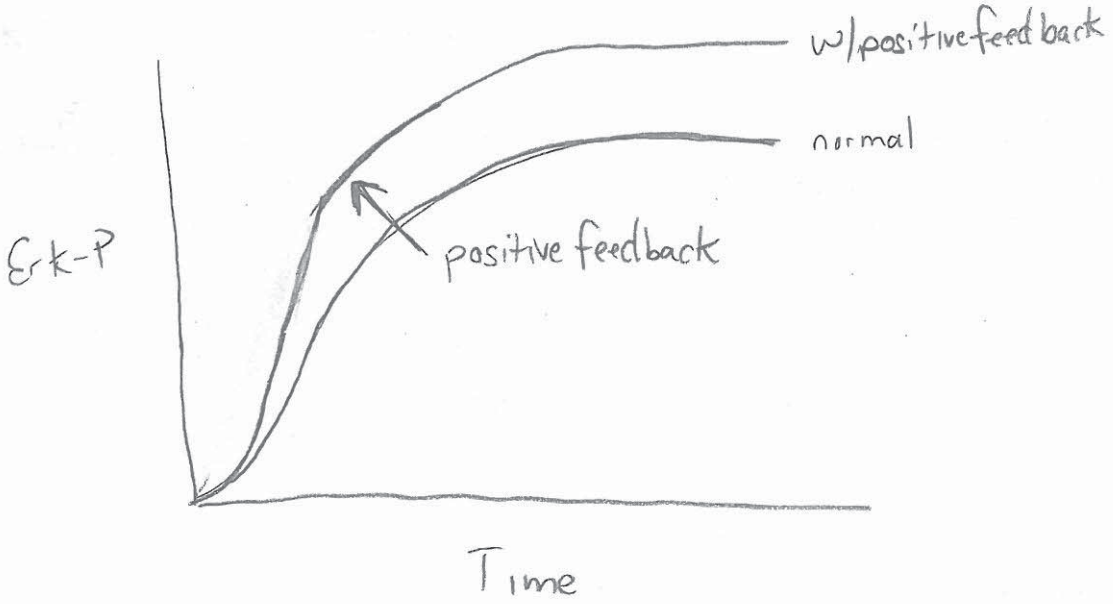
$$Erk\text{-}P + T \underset{k_{19}}{\overset{k_{18}}{\rightleftharpoons}} Erk\text{-}P:T \xrightarrow{k_{20}} Erk\text{-}P + T_P$$

$$T_P + Erk \underset{k_{22}}{\overset{k_{21}}{\rightleftharpoons}} Erk:T_P \xrightarrow{k_{23}} Erk\text{-}P + T_P$$

Now:

$$\frac{d[T]}{dt} = k_5[TR][L] - k_6[T] - k_7[T][ERK] + k_8[T:ERK] + k_9[T:ERK] - k_{18}[Erk\text{-}P][T] + k_{19}[Erk\text{-}P:T]$$

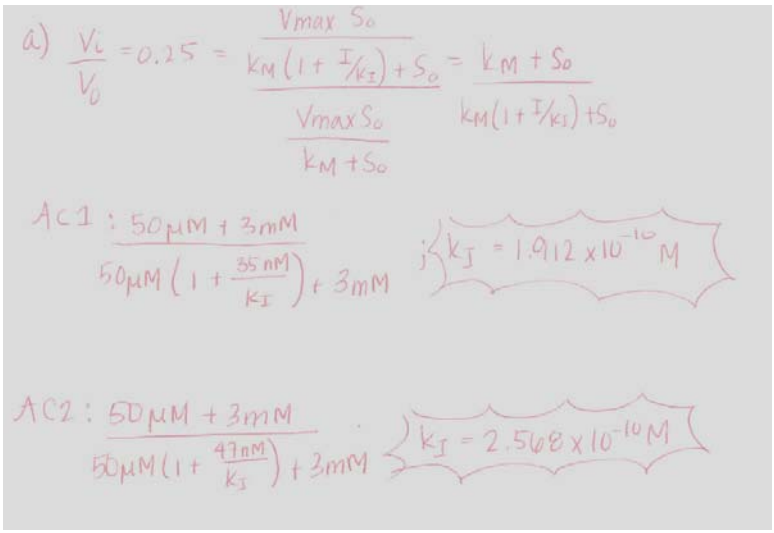
$$\frac{d[Erk\text{-}P]}{dt} = k_9[T:ERK] - k_{10}[Erk\text{-}P][Ptase] + k_{11}[Erk\text{-}P:Ptase] - k_{18}[Erk\text{-}P][T] + k_{19}[Erk\text{-}P:T] + k_{20}[Erk\text{-}P][T] + k_{23}[Erk:TP]$$



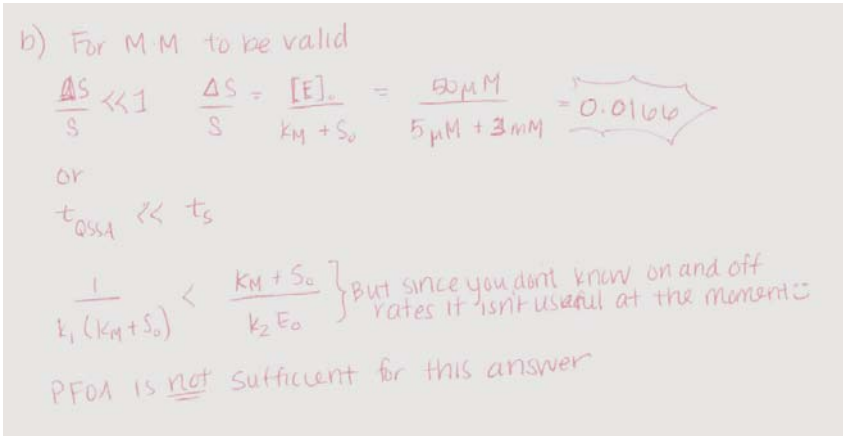
Question 3. (26 points total)

BigPharmaCo. has recently developed two new ATP-competitive inhibitors for the SRC tyrosine kinase that they are calling AC1 and AC2. An in vitro kinase test has been performed with 3 mM ATP, 50 micromolar SRC, and 1 mM p130Cas, a SRC substrate with 5 micromolar Km. In this test, AC1 has demonstrated an IC25 of 35 nM, while AC2 has an IC25 of 47 nM (note that IC25 is defined as the inhibitor concentration at which 25% of the activity is remaining).

A) (6 points) Calculate the Ki value for each AC1 and AC2. Assume Km for SRC-ATP is 50 micromolar.



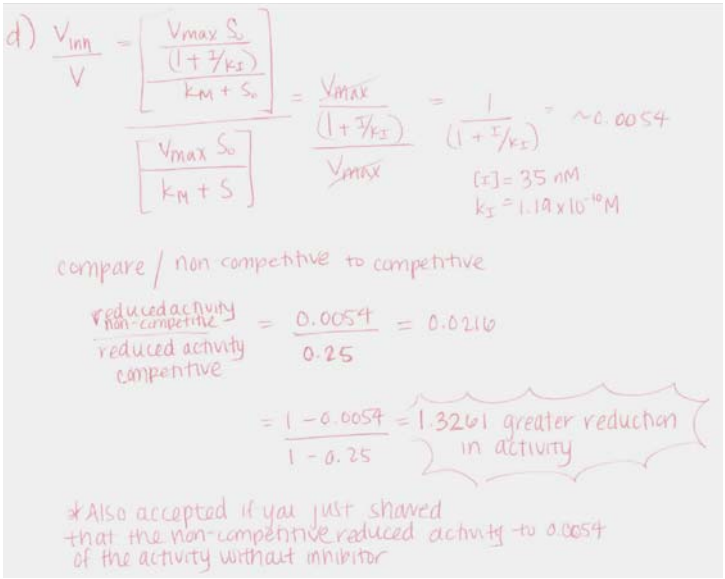
B) (7 points) You want to model this reaction, but are unsure if the reaction conditions satisfy the assumptions necessary for the Michaelis-Menten approximation. In the absence of inhibitor, does this in vitro kinase test meet the criteria for M-M kinetics? Provide a quantitative answer.



C) (7 points) Does the presence of the AC1 inhibitor at IC25 alter your answer? Provide a quantitative answer.

Michaelis-Menten is still valid for the original substrate so no, the answer shouldn't change. You can still assume that $\Delta S/S << 1$. However, if you were anylyzing the depletion of ATP as a substrate, this might not hold since the effective K_M has changed for ATP binding.

D) (6 points) As an ATP analog, might be better to re-design AC1 to be a non-competitive inhibitor (e.g. re-factor to be a Type II inhibitor). Assuming that the Ki does not change after refactoring, how much has the inhibitor improved? In other words, at the same concentration of inhibitor, how much has the reaction been inhibited by the competitive vs. non-competitive inhibitor?



Question 4. (20 points total)

Short answers – one to three sentences.

a) (4 points) Strong positive feedback is potentially very dangerous for the cell, yet is critical for selected biological processes. Name one of the these processes and describe the role of strong positive feedback.

The example we covered in class is during development, strong positive feedback allows a cell to continue through to its differentiated state, even though the input signal may shut off (a cell stopping halfway through differentiation would not be effective). Many other examples from biology are appropriate here.

b) (4 points) To calculate ΔH from an ITC experiment, does it matter if multiple injections are performed simultaneously? Explain.

ΔH is a state function (not a state experiment – experiments don't have states!) , so you could get this value by performing all injections simultaneously.Multiple injections are useful to curve fit for Kd, and also to give more data points to better constrain deltaH. However, injecting all at once will result in the same cumulative value of heat released.

c) (4 points) As discussed in class, protein microarrays can be used to establish Kd values for a large number of combinations of interactions. Is it possible to use mass spectrometry to generate this same sort of data? Justify your answer.

Yes, you could envision a way in which (as long as the complex is not sensitive to the ionization process) you could add in different amounts of ligand, and do MS to see how much complex is formed, and use this titration curve to get Kd (in fact, this is often done for small molecules).

d) (3 points) Name three ways the cell regulates the catalytic rate of a given enyzme.

The three covered in class were:
1.) Transcription/Translation
2.) Localization

3.) Post-translational modification

Many answers were acceptable, as long as it was correct/justified if not obvious how it would work.

e) (5 points) Explain how scaffolding proteins can both promote downstream signaling and prevent it from reaching maximal signals. Cite examples from class where relevant

In Particular, we covered Ste5 as a yeast scaffold which binds ste7, ste11, and fus3. Ste5 can promote downstream signals by bringing all of the necessary kinases in proximity of each other, and can prevent downstream signal form reaching maximal signals by binding tightly to the phosphorylated form of fus3, or by in low concentrations of kinase, having one kinase bound to each scaffold so few scaffolds have all three kinases bound. Note, ste5 by itself is NOT a kinase!

20.320 Analysis of Biomolecular and Cellular Systems
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