

20.320 — Exam #1

November 27, 2010

Question 1 (25 points total)

In your job at BigPharmaCo. you need to test a panel of small molecule inhibitors (each approximately 500 Da) for their ability to inhibit EGFR (185 kDa) and their specificity against a broad panel of kinases. Given the exorbitant cost of in vitro kinase assays for this panel of kinases, you decide that it might make more sense to gather some biophysical data first prior to characterizing their effect on enzymatic activity. First, you need to figure out how well each of these inhibitors binds to the EGFR target protein.

- a) (4 points) Design an SPR experiment to test the interaction affinity between EGFR and the first compound in the series. How will you get the desired information out of your experiment?
- c) (6 points) For the first compound, you determine the following parameters: $k_{on} = 10^5 \text{ molM}^{-1} \text{ sec}^{-1}$ and $k_{off} = 10^{-3} \text{ sec}^{-1}$. For these parameters and your experimental design, how long will it take for each analysis to reach 75% RUeq, using a ligand concentration of 50 nM?
- d) (4 points) One of the major problems with SPR is non-specific binding to the surface of the chip. How can you identify this problem, and how can you correct data that has already been gathered?
- e) (5 points) To design the next generation of improved inhibitors you need to know the thermodynamic parameters of the various inhibitors. Describe an experimental setup, including the concentrations of the various components, to generate this information for the first compound, given the parameters in part c, above. Experimental design is sufficient - no need to work through equations, although you should provide the relevant equations.

Question 2 (21 points total)

BCR-Abl is a constitutively active fusion tyrosine kinase associated with oncogenesis and progression of chronic myeloid leukemia. One of the first structure-based designed kinase inhibitors is imatinib, an ATP-analog inhibitor of BCR-Abl. K_m for BCR-Abl interaction with ATP is 50 micromolar, and K_i for imatinib is 22 nM. ATP concentration in the cell is 4 mM.

- a) (6 points) Since BCR-Abl is such a potent driver of leukemia, 99% inhibition is desired to slow down disease progression. Calculate the imatinib concentration required for this level of inhibition.
- b) (4 points) Provide 2 reasons why almost all kinase inhibitors are ATP-analogs.
- c) (6 points) Given the difficulty in competing with ATP, BigPharmaCo, Inc. has recently started a program to develop a novel, non-competitive inhibitor targeting BCR-Abl. Unfortunately, the first inhibitor ($K_i = 50$ nM) generated in this program is limited by solubility issues to a concentration of 1.2 micromolar in the cell. How effective will this inhibitor be given this concentration limitation?
- d) (3 Points) Describe, in 2 sentences or less, the mechanism of action of Type II inhibitors.
- e) (2 points) Why are Type II inhibitors not applicable to BCR-Abl?

Question 3 (22 points total)

As discussed in class, Ste7 is unable to phosphorylate Fus3 unless the Ste5 scaffold is present. Activated Fus3 phosphorylates the Ste5 scaffold, thereby increasing the affinity of the interaction between phosphorylated Fus3 and Ste5.

- a) (5 points) Draw a schematic diagram of this 3-component signaling cascade. You can start with activated Ste7. Assume Ste7 binds to Ste5 first, followed by Fus3. Assume the order of dissociation is the inverse of the binding order (e.g. Fus3 unbinds from Ste5 first).
- b) (8 points) Write out ODEs for each species.
- c) (5 points) While activated Fus3 can drive proliferation, activated Kss1 drives filamentous growth. Given that these are both MAP kinases that share a large number of substrates, provide two potential hypotheses explaining how the biological response can be so different downstream of the activation of these two kinases.
- d) How does the increased affinity between Fus3 and phosphorylated Ste5 affect the biological activity of this system? Provide a mechanism underlying your answer (short answer, 1-2 sentences).

Question 4 (20 points total)

Jim Smith, a recent hire at BigPharmaCo. in their process engineering group, needs to set up a reactor to make a large supply of bioactive peptides. The process currently used at BigPharmaCo begins with recombinant proteins. Each protein is converted to 2 bioactive peptides in a single step using ClpX, a proteolytic complex. This reaction requires energetic input: ClpX consumes one ATP to cleave each protein. You can assume independent binding (i.e. no cooperativity) for ClpX binding to ATP or to the recombinant protein.

- a) (4 points) Provide the chemical equations for this reaction, given the following rate parameters for ClpX-protein: $k_1 = 2 \times 10^4 \text{ L mol}^{-1} \text{ sec}^{-1}$, $k_{-1} = 1 \times 10^{-3} \text{ sec}^{-1}$, $k_2 = 2 \times 10^{-3} \text{ sec}^{-1}$. For ClpX-ATP: $k_1 = 5 \times 10^3 \text{ L mol}^{-1} \text{ sec}^{-1}$, $k_{-1} = 10^{-2} \text{ sec}^{-1}$, $k_2 = 2 \times 10^{-2} \text{ sec}^{-1}$.
- b) 10 points) Given a 10L reactor with 5 micromolar ClpX, 20 mM ATP and 100 mM protein, calculate how long it will take for the reactor to reach steady state.
- c) (6 points) How long will the reactor run at maximal efficiency, and how much product will be generated by the reactor during optimal operation?

Question 5 (14 points total)

Short answers - one to three sentences.

- a) (4 points) Name two ways to test the biological role of a given phosphorylation site.
- b) (4 points) How does strong negative feedback affect the duration and amplitude of the MAPK signaling network output? How does this differ for strong positive feedback?
- c) (2 points) Name 2 human MAP kinases.
- d) (4 points) Name two reasons why quantitative interaction affinities generated by protein microarrays may not be applicable to modeling reactions in cells.

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