20.320 Exam 1
Thursday October 4th, 2012
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. (25 points total)

Bromodomains are protein domains that bind to acetylated lysines (similar to SH2-phosphotyrosine interactions). Jay Bradner at Harvard has recently developed JQ-1, a cell-permeable small molecule (mass: 642 g/mol) that binds and inhibits a subset of bromodomains. In the lab, you would like to determine the affinity between JQ-1 and BrdX (mass: 24,354 g/mol), your bromodomain of choice.

A) (9 points) Design an SPR experiment to quantify the interaction affinity between JQ1 and BrdX, including proper controls and which molecule is immobilized. The details of exactly how SPR works are not critical to answer this question. Justify your immobilization selection by calculating the number of captured molecules needed to obtain a 10 RU signal change in both configurations. (Note: assume a 10 mm^2 chip surface area).

B) (10 points) Given the following parameters:

$k_{on} = 3x10^4$ L mol^{-1} s^{-1}

$k_{off} = 4x10^{-2}$ s^{-1}

How long will it take to complete the experiment using a ligand concentration of 200 x 10^{-9}M, assuming the association reaches 90% of its equilibrium value before ligand stops and 90% of the associated ligand dissociates by the end of the experiment?

C) (6 points) Although SPR is a very powerful approach, it does have some limitations. Provide two limitations and describe how you would know that they are affecting your measurements.

Question 2. (25 points total)

Overexpression or mutation of the Epidermal Growth Factor Receptor (EGFR) has been associated with a broad range of cancers. As discussed, there are many companies that have developed molecularly-targeted therapeutics, and several of these compounds are ATP analogs designed to inhibit EGFR. One of these compounds, Gefitinib, is currently approved for lung cancer patients with high EGFR expression or with selected activating mutations in EGFR. Km for the EGFR-ATP interaction is 50×10^{-6} M, kcat for EGFR-ATP is 10^6 sec-1, and K_i for the EGFR-Gefitinib interaction is 22×10^{-9}M. ATP concentration in the cell is 3×10^{-3}M.

a) (10 points) Due to dose-limiting toxicity, the intracellular concentration of Gefitinib is limited to 100×10^{-9}M. How effective is this dose at shutting down EGFR activity?

b) (4 points) How much does EGFR inhibition change in cells expressing 10^6 copies of EGFR versus cells expressing 10^4 copies of EGFR?

c) (4 points) Why is a strong increase in expression of EGFR often associated with cancer, even in the absence of ligand stimulation?

d) (3 points) Describe how the mechanism of action of a Type II inhibitor differs from that of an ATP analogue.

e) (4 points) Given a cancer cell line with an activating EGFR mutation, would you use a Type II inhibitor or an ATP analogue inhibitor and why?

Question 3. (35 points total)

As discussed in lecture, the mitogen-activated protein kinase (MAPK) cascade is ultrasensitive, demonstrating very strong response to low-level input. Many other signaling systems also feature ultrasensitivity. One of these is the CDK1 (kinase) - CDC25 (phosphatase) interaction, which regulates the cell cycle. In a recent manuscript (PNAS, 2012), Lu et al. developed a computational model describing multi-site phosphorylation of CDC25 by CDK1, where the multiply-phosphorylated CDC25 phosphatase is fully active. For simplicity in this model, assume 2 CDK1-dependent phosphorylation sites on CDC25. In the parts that follow, please designate CDK1 as A and CDC25 as B and use the nomenclature from class to describe complexes and phosphorylated species (BP is B that's been phosphorylated once, BPP is double-phosphorylated). Remember to state your assumptions!

A. (6 points) Write out the chemical reactions for the above system. You can start with phosphorylated, activated CDK1.

B. (12 points) Write out ODEs for each species.

C. (4 points) What are the key points in this system that lead to ultrasensitivity?

D. (3 points) From a design perspective, how could you increase the responsiveness of the system?

E. (10 points) One of the substrates of CDC25 is the CDK1 kinase. Add this reaction into your system and add in the additional ODE(s) reflecting this reaction. What biological principle does this introduce into the system and why is it useful.

Question 4. (15 points total)

Short answers – one to three sentences.

a) (3 points) Describe what information you can obtain from ITC and not from SPR and vice versa.

b) (3 points) How does strong positive feedback affect the duration and amplitude of the kinase signaling cascade?

c) (3 points) Name one reason why the quantitative interaction affinities and kinetics generated by surface plasmon resonance (SPR) may not be applicable to modeling reactions in cells.

d) (3 points) Describe the pseudo first order approximation (PFOA). Is this applicable in ITC and SPR? Why or why not.

e) (3 points) Describe one technique that can be used to determine if two proteins interact in vitro.

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