University of Toronto
Faculty of Applied Science and Engineering

Final Examination, April 25, 2001

Fourth year – Program 05

BME496S – Cellular Bioengineering

Exam type: C

Examiner – P.W. Zandstra

<u>Instructions:</u> Read the whole exam before attempting any of the questions. Answer only one of questions 3 and 4.

Good Luck!

1. *[20%]* After years of work in cancer biology, a keen graduate student isolated a mutant erythropoietin (EPO) receptor deficient in endocytosis. As a final step in her Ph.D., her supervisor asked her to determine if the mutant EPOR might be useful for stimulating proliferation in a cell line (J007) that had a very high complex activation threshold ($C_{threshold}$). In fact, previous studies had shown that the J007 cell line only proliferated when >75% of the total receptors were occupied. The student transfected the mutant receptor into the J007 cell line (which originally did not express any EPO receptors) and isolated two clones, expressing 65,000, and 745,000 mutant EPO receptors per cell.

   i) Assuming that the K_D of the mutant receptor is 1 nM, and that the experiment was performed at a cell density (ρ) of 3×10^6 cells/mL, determine the ligand concentration above which cell proliferation may be observed.

   ii) How would your results change if it was found that the receptor required dimerization for signaling?

   iii) Develop an expression that links cell proliferation to complex activation threshold. Would your results change if the receptor was capable of internalization? Why or why not?

2. *[20%]* It has been recently shown that many types of cells migrate othokinetically in response to gradients of growth factors for which they have cell surface receptors.

   i) Sketch a single cell migrating orthokinetically up a concentration gradient. Indicate on your diagram the cell's migration path and the gradient direction.

   ii) Indicate how the linear translation speed (S) and the persistence time (P) could be determined from your diagram.

   iii) Propose a model, based on receptor ligand binding that is consistent with average displacement of the cell up the gradient and the "random" changes in cellular direction that occur during cell migration.

   iv) Suggest how the information you have determined for the one cell you are studying may be used to obtain information about the properties of a population of such cells.

NOTE: PLEASE CHOOSE EITHER QUESTION 3 OR 4.
DO NOT DO BOTH

3. *[20%]* The introduction of genes to cells to permanently change their genotype has a very large clinical potential. Despite significant efforts, success in this technology has been limited. A major reason for this may be our insufficient understanding of the barriers to gene delivery.

   i) Using a diagram, outline what you predict are 3 major barriers (of the ~7 that were discussed in class) to gene delivery. In one sentence justify why you have chosen these barriers.

   ii) Develop realistic equations for the processes that govern the barriers you have chosen. Define all parameters and give units consistent with your equations.

   iii) Describe how you would experimentally determine the relevant parameters for 2 of the 3 barriers you have chosen. Ensure that the results of your experiments are consistent with the units you have outlined above.

4. *[20%]* Consider a paracrine cell system, in which macrophages secrete a ligand that can bind to a surface receptor expressed by lymphocytes. Binding occurs via a simple one-step rate process characterized by association rate constant k_f and dissociation rate constant k_r; complexes are internalized by a first order process with endocytic rate constant k_{eC}, while free receptors are internalized by a first order process with endocytic rate constant k_{eR}; recycling of ligands, receptors and complexes is negligible. Receptors are synthesized by the lymphocytes at a rate V_R, and ligand is secreted by the macrophages at a rate V_L.

   i) Write the appropriate transient mass action kinetic equations governing the number of free receptors (R) and complexes (C) on the lymphocytes – and the concentration of ligand in the extracellular medium (L).

   ii) In the absence of the macrophages, what is the steady state value of R_{TB} – the total number of cell surface receptors expressed by the lymphocytes.

   iii) In the presence of the macrophages, what are the steady state values of C and L?

   iv) Under what conditions (i.e., parameter regimes) will the ligand continually accumulate in the extracellular medium instead of reaching a steady state?

5. *[20%]* Stem cell factor (SCF) is an interesting cytokine that can be presented to cells either in soluble form, or as a cell surface bound ligand. The activity of stem cell factor depends on its ability to bind its receptor (*c-kit*) before it is bound and inactivated by a soluble inhibitory ligand (Inhibition Factor, IF). Interestingly the intrinsic forward rate constant, k_{on}, is 20% smaller for IF binding SCF in solution than SCF binding to its cell surface receptor. The diffusion coefficient of SCF in solution is $\sim 10^{-7}$ cm^2/s, and you may assume that the encounter radius, s, is 10 nm, the cell radius, a, is 10 μm, and all other parameters are constant.

   i) In the special case that the reverse rate constants, k_r are the same ($\sim 3.4 \times 10^{-1}$ min^{-1}) for both interactions (SCF:*c-kit* and SCF:IF) -- i.e., k_r is not effected by diffusion or receptor expression -- the ability of SCF to overcome IF and activate its receptor is essentially governed by the overall forward rate constant (k_+) of each binding reaction. Determine the critical number of receptors that a responsive cell should express in order for the K_D of the SCF:c-kit interaction to be the same as the K_D of the SCF:IF interaction. You may express your solution in terms of variables, or solve the problem using typical parameters.

   ii) How would your result change if k_r was effected by diffusion and receptor expression?

6. *[20% total]* Short answer questions.

<u>You should spend a maximum of 7.5 min. on each of these questions</u>

A. [5%] i) Distinguish between multipotent, totipotent and pluripotent stem cells; ii) list 2 properties of stem cells that distinguish them from progenitor cells.

B. [5%] In class we defined three scenarios where ligand mediated receptor down-regulation could occur. List two of the three scenarios. For one of the two you have listed, develop an expression where a ratio of trafficking parameters allows you to evaluate whether or not ligand mediated receptor down-regulation will occur.

C. [5%] The paper "Bioengineering models of cell signaling" by Asthagiri & Lauffenburger describes four modes that cells can respond (adapt) to step changes in a signal. Briefly summarize three of the modes presented and suggest how two of them can be mathematically described.

D. *[5%]* For each of the below diagrams rank the relative adhesive strength of all the cell – cell and cell – surface interactions. How might this phenomenon be used for tissue engineering?