Solutions to PS2
March 1, 2015

Problem 1

(a) The elementary reactions are the following:

- binding reaction at the surface

$$R_s + L_s \rightleftharpoons C_s \tag{1}$$

- internalization of surface species with the internalization of free ligand assumed to be negligible

$$R_s \rightarrow R_i \tag{2}$$
$$C_s \rightarrow C_i \tag{3}$$

- binding and unbinding within endosomes

$$R_i + L_i \rightleftharpoons C_i \tag{4}$$

- recycling of internal species

$$R_i \rightarrow R_s \tag{5}$$
$$C_i \rightarrow C_s \tag{6}$$
$$L_i \rightarrow L_s \tag{7}$$

- degradation of internalized species

$$R_i \rightarrow \emptyset \tag{8}$$
$$C_i \rightarrow \emptyset \tag{9}$$
$$L_i \rightarrow \emptyset \tag{10}$$

- synthesis of new receptors

$$\text{building blocks} \rightarrow R_s \tag{11}$$

The time-dependent state variables are the surface and internal free receptor (R_s and R_i, respectively), the surface and internal ligand-bound receptors (C_s and C_i) and free ligand at the surface and within endosomes (L_s and L_i).

Since both ligand and receptor are degraded, neither species is conserved. Therefore, no mass balances can be written. Unlike the ligand, receptor is both synthesized and degraded. Therefore, the amount of receptor in the system is expected to reach a non-zero, steady-state value for a particular system condition.

The time evolution of the state variables are governed by the following mass-action rate equations:

$$\frac{\mathrm{d}R_s}{\mathrm{d}t} = -k_f L_s R_s + k_r C_s - k_{eR} R_s + k_{rec}(1 - f_R) R_i + V_s \tag{12}$$

$$\frac{\mathrm{d}C_s}{\mathrm{d}t} = k_f L_s R_s - k_r C_s - k_{eC} C_s + k_{rec}(1 - f_C) C_i \tag{13}$$

$$\frac{\mathrm{d}L_s}{\mathrm{d}t} = \eta^{-1} \left(-k_f L_s R_s + k_r C_s + k_{rec}(1 - f_L) L_i\right) \tag{14}$$

$$\frac{\mathrm{d}R_i}{\mathrm{d}t} = k_{eR} R_s - k_f^{'} \left(L_i/\beta\right) R_i + k_r^{'} C_i - k_{rec}(1 - f_R) R_i - k_{deg} f_R R_i \tag{15}$$

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = k_{eC} C_s + k_f^{'} \left(L_i/\beta\right) R_i - k_r^{'} C_i - k_{rec}(1 - f_C) C_i - k_{deg} f_C C_i \tag{16}$$

$$\frac{\mathrm{d}L_i}{\mathrm{d}t} = -k_f^{'} \left(L_i/\beta\right) R_i + k_r^{'} C_i - k_{rec}(1 - f_L) L_i - k_{deg} f_L L_i \tag{17}$$

where all parameters are defined in the problem statement, including the receptor synthesis rate V_s with units of # receptors/min/cell. We have introduced $k_f^{'}$ and $k_r^{'}$ for the binding and unbinding rate constants within endosomes.

Special attention must be given to units. First, note that L_s is given in molar units whereas the terms on the right side of equation (14) have units of #/cell. Thus, the rhs of this equation must be converted into a molar quantity. The factor η^{-1} converts #/cell into a molar quantity where $\eta = N_{Av}/n$ where n is the volume density of cells. Second, note that L_i is measured in #/cell but that $k_f^{'}$ and $k_r^{'}$ have the same units as their extracellular counterparts. Thus, it is necessary to convert the units of L_i into a molar quantity. To do so, we assume that the volume of endocytic vesicles is 2% of the total cell volume (10^{-14} L/cell) (Zigmond et. al. (1982) *J. Cell. Biol.*) and use the conversion factor $\beta = 10^{-14} N_{Av}$.

(b) When no ligand is present, the species L and C do not exist, and the mass-action kinetic equations are:

$$\frac{\mathrm{d}R_s}{\mathrm{d}t} = -k_{eR} R_s + k_{rec}(1 - f_R) R_i + V_s \tag{18}$$

$$\frac{\mathrm{d}R_i}{\mathrm{d}t} = k_{eR} R_s - k_{rec}(1 - f_R) R_i - k_{deg} f_R R_i \tag{19}$$

At steady-state, Eqs. 18 and 19 can be set to 0 as follows,

$$-k_{eR} \bar{R}_s + k_{rec}(1 - f_R) \bar{R}_i + V_s = 0 \tag{20}$$

$$k_{eR} \bar{R}_s - k_{rec}(1 - f_R) \bar{R}_i - k_{deg} f_R \bar{R}_i = 0 \tag{21}$$

and the sum of these equations gives

$$k_{deg} f_R \bar{R}_i = V_s \tag{22}$$

where $\bar{R}_s$ and $\bar{R}_i$ denote steady-state values. Using the parameter values provided in the problem statement, one can readily solve for $\bar{R}_i$. Then, using Eq. 20 set to 0, we can solve for $\bar{R}_s$. The solutions are $\bar{R}_i = 100/\left(0.2 \cdot 0.002\right) = 2.5$ x 10^5 receptors/cell and $\bar{R}_s = \left((0.06 \cdot 0.8 + 0.002 \cdot 0.2) \cdot 2.5 \text{ x } 10^5\right)/0.03 \approx 10^6$ receptors/cell.

(c) We use the steady-state values of R_i and R_s computed in the part (b) as initial conditions. The initial values of C_s, C_i and L_i are 0, and the initial value of $L_s = 1$ nM.

All necessary parameter values are provided in the problem set except $k_f^{'}$ and $k_r^{'}$. We approximate that binding is less effective at endocytic pH with $k_f^{'} = 0.1 k_f$ and unbinding is more rapid with $k_r^{'} = 2k_r$.

The differential equations (12)-(17) are simulated in Matlab to predict the time evolution of the system (see attached code). The plots below summarize the results, and if desired, the total amount of ligand inside the cell can be calculated by

$$L_i^{\#} = L_i + C_i. \tag{23}$$

Problem 2 (a) Equation (1) in the problem statement is an increasing monotonic function of substrate concentration. Thus, $\dfrac{\mathrm{d}P}{\mathrm{d}t}$ is at its maximum value when S is at its maximum value. Since substrate is converted to product, the concentration of substrate is greatest at initial time.

(b) The maximum velocity occurs when the function given by equation (2) in the problem statement reaches its saturation value. This occurs when S_o is large relative to K_m at which point the velocity becomes $V = V_{\max} = k_2 E_o$.

Figure 1: Time evolution of receptor species upon stimulation with 1 nM EGF (Problem 1)

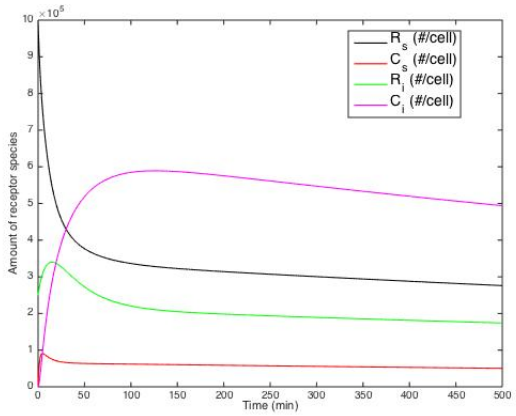

Figure 2: Free internal ligand upon stimulation with 1 nM EGF (Problem 1)

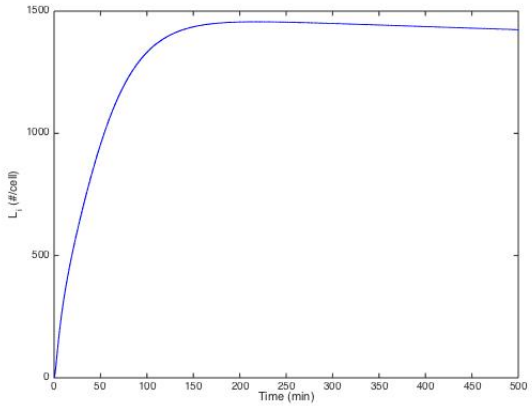

Figure 3: Depletion of surface ligand in response to 1 nM EGF (Problem 1)