Problem Set 7

1 Design principles (22 points)

1.1 Robustness

Robust timing: Uri Alon Exercise 8.4 (8 points)

1.2 Optimality

a. Optimal genetic code: Uri Alon Exercise 9.4 (8 points)

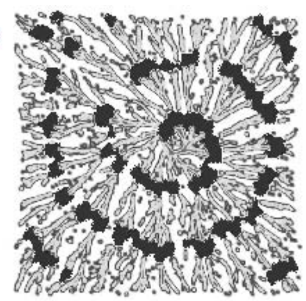
b. Fitness function: Uri Alon Exercise 10.1 & 10.2 (6 points)

2 cAMP system (28 points)

Dictyostelium amoebae are free living cells with a remarkable twist: under the stress of starvation, large numbers of amoebae are able to collect together to form a single multi-cellular organism (Fig. 1). The entire process begins when starving amoebae emit pulses of the chemoattractant cAMP, inducing the surrounding cells to move in their direction and to secrete cAMP themselves. This process generates outgoing spiral waves of cAMP which direct the entire population towards the original source (Fig. 2). We will try to understand the origin of and the response to these cAMP waves.

Dictyostelium life cycle removed due to copyright restrictions.
Please see http://www.dictyostelium.com/devcyc.gif.

Fig. 2



Problem 2 courtesy of Alexander van Oudenaarden. Used with permission.

2.1 Chemical kinetics of cAMP signaling (6 points)

Two species of cAMP receptors exist in the Dictyostelium cell membrane: an 'activator' A, and an 'inhibitor' I, both of which act on a third protein R. When bound to cAMP, a pair of A molecules catalyzes the conversion of R to an active form R^*, and a pair of I molecules catalyzes the reverse reaction (Fig. 3).

a. The initial binding of cAMP, C, to its receptors is described by:

$$A + C \underset{k_A^-}{\overset{k_A^+}{\rightleftharpoons}} AC \qquad\qquad I + C \underset{k_I^-}{\overset{k_I^+}{\rightleftharpoons}} IC$$

Write down equations describing the time evolution of $[AC]$ and $[IC]$. Assume that $[AC] \ll [A_{tot}]$, and $[IC] \ll [I_{tot}]$; let a be proportional to $[AC]$, i to $[IC]$, and c to $[C]$. By making a convenient choice of units, show that these equations can be written in the form

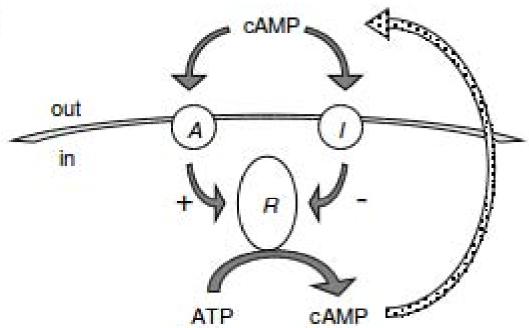
$$\frac{da}{dt} = k_A^-(c - a) \qquad\qquad \frac{di}{dt} = k_I^-(c - i). \tag{1}$$

b. The reactions involving activation and inactivation of R reach a rapid equilibrium:

$$R \underset{k_R^- i^2}{\overset{k_R^+ a^2}{\rightleftharpoons}} R^*$$

The a^2 and i^2 terms arise because it takes two molecules of AC or IC to catalyze these conversion reactions. Setting $\beta = \frac{k_R^-}{k_R^+}$, find an expression for the rapid equilibrium value of $r = [R^*]$ in terms of a, i, and $[R_{tot}]$.

Fig. 3



2.2 Positive feedback and oscillations (12 points)

The molecule R is an enzyme known as adenylate cyclase, which in its active form catalyzes the conversion of ATP into cAMP in the cytoplasm. The presence of extracellular cAMP thus stimulates the synthesis of intracellular cAMP, which in turn is secreted into the environment, creating a positive feedback loop (Fig. 3). Let $c_1 = [\text{cAMP}_{in}]$; let the rate of cytoplasmic cAMP synthesis be $k_1 r$; and let the rate constant for its secretion be k_0. $cAMP$ is continuously degraded by phosphodiesterase enzymes both inside and outside the cell, with rate constants γ_1 and γ_0, respectively. The entire network is described by the following equations:

$$\frac{dc_1}{dt} = k_1 r - (\gamma_1 + k_0)c_1 \qquad\qquad \frac{dc}{dt} = k_0 c_1 - \gamma_0 c. \tag{2}$$

a. Assuming that the concentrations c_1 and a reach rapid equilibrium, reduce the four equations in (1) and (2) to two equations for the slow variables c and i. Show explicitly the choice of units required to produce the simplified form shown in (3). What is the value of k?

$$\frac{dc}{dt} = \frac{c^2}{\beta i^2 + c^2} - c \qquad\qquad \frac{di}{dt} = k(c - i). \tag{3}$$

b. When *Dictyostelium* is grown in liquid medium, extracellular cAMP is well stirred, making its concentration uniform over space. However, under such conditions, the cAMP concentration is known to oscillate over time. Find the conditions on k and β so that the system is oscillatory (we are talking about limit cycle oscillations here).

c. **COMPUTATION** On a graph of i vs. c, plot the nullclines and simulate the time evolution of the system for an oscillatory case.

d. Suppose the system only required a single molecule of AC or IC in order to catalyze the R conversion reactions. Comment on the behavior of the system in this case.

2.3 COMPUTATION Diffusion and cAMP waves (10 points)

When cells are grown on a plate, cAMP diffusion is slow, and the extracellular cAMP concentration is no longer uniform. Consider a plate on which there exists a uniformly distributed population of cells. Each cell senses cAMP in its environment, and secretes fresh cAMP in response. This new batch of cAMP is able to reach neighboring cells, stimulating them to synthesize more cAMP, and so on. The situation is similar to one in which a number of radio transmitter towers (cells) are used to detect, amplify, and re-broadcast a weak radio signal (cAMP). The cAMP concentration now varies over space as well as time. For simplicity, we will analyze a 1-dimensional case, with cells uniformly distributed along a line. We can assume that the cells have fixed positions over the timescales considered, because their chemotaxis is relatively slow. This system obeys the equations

$$\frac{\partial c}{\partial t} = \frac{c^2}{\beta i^2 + c^2} - c + D\frac{\partial^2 c}{\partial x^2} \qquad\qquad \frac{\partial i}{\partial t} = k(c - i). \tag{4}$$

In this problem you will have to develop a numerical code for simulating these equations[1]. The system is assumed to extend from $x = -1$ to $x = +1$, with no flow at the boundaries. As for the initial conditions, consider a pulse of cAMP centered in the origin, that goes to zero towards the boundaries and that has a typical width σ (try to use a smooth but localized function); assume an uniform initial concentration of inhibitor i_o.

a. Use the following parameters: $\beta = 4$, $k = 0.5$, $D = 10^{-7}$, $\sigma = 0.1$, and $i_o = 0.1$. Run the simulation to see the emergence of cAMP waves emanating from the origin. Plot a typical cAMP profile, indicating the direction of motion of the waves.

b. Run the simulation again, this time with a k value which produces a non-oscillating system. Describe the typical cAMP profile once the transients have died out. Can cells find the initial source of cAMP based on this type of profile?

c. A simple concentration gradient would allow cells to find the cAMP source. Why do you think *Dictyostelium* uses waves of cAMP rather than a gradient in order to trigger cell aggregation?

Feedback (+1 Extra Credit)

[1] Page 76-78 of the supplementary notes by Alexander van Oudenaarden under Reaction-Diffusion models in the materials section.