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## Structural Adaptation and Robustness of *Dictyostelium* Ligand-Receptor Kinetics for Low and High Ligand Concentrations<sup>†</sup>

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### SUMMARY

Ligand-receptor interactions are responsible for adaptation and robustness of all cellular life to most chemical external stimuli, and are mediated by cellular networks whose structure appears to be highly conserved among different organisms. Although many ligand-receptor networks exhibit a common structure, the dynamic response to variations in the ligand concentration can be vastly different from network to network. This suggests that certain parameters of the network have evolved by nature to provide appropriate performance and robustness characteristics for different situations. We investigate the system's response in the cases of low and high concentrations of external cAMP, corresponding to two distinct stages of the *Dictyostelium* life cycle. Our analysis reveals highly robust responses from the ligand-bound receptor kinetics for low ligand concentration, and such high levels of robustness are likely to be required from each individual *Dictyostelium* cell to survive this stage of its life cycle. We show that overshoot is prohibited by the structure of network regardless of the kinetic constants values, and the particular values chosen in the original model are shown to lead to a critically damped response. On the other hand, for high ligand concentrations an extreme reduction in the magnitude of the network response to external signals is observed, and this may be responsible for the completely different physiological behaviour of the organism as groups of up to  $10^5$  *Dictyostelium* cells aggregate to form a slug. The receptor/ligand interaction network may have evolved to provide an optimal trade-off between maximising the speed of response and prohibiting overshoot as it follows external oscillatory signals. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: *Dictyostelium*; cAMP oscillations; Robustness

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## 1. Introduction

In cellular signal transduction, external signalling molecules, called ligands, are initially bound by receptors which are distributed on the cell surface. The ligand-receptor complex then initiates the response of various intracellular signal transduction pathways, such as activation of immune responses, growth factors, etc. Inappropriate activation of signal transduction pathways is considered to be an important factor underlying the development of many diseases. Hence, robust performance of ligand and receptor interaction networks constitutes one of the crucial mechanisms for ensuring the healthy development of living organisms.

In [1], a kinetic model for how the distribution of chemo-receptor complexes affects the cell response was developed from time series responses to perturbations in ligand concentration. By analysing this model it was identified that the distribution of complex size in the membrane depends on the receptor free energy. Physical details about ligand-receptor interactions are discussed in [2]. In [3], the authors proposed the existence of a generic structure for ligand-receptor interaction networks, and developed a corresponding general model for these types of networks. This model suggests that the ability to capture ligand together with the ability to internalise bound-ligand complexes are the key properties distinguishing the various functional differences in cell receptor kinetics. The above studies have highlighted the fact that striking structural similarities exist between the various different types of ligand-receptor interaction networks found in nature. It is also tempting to speculate that nature will have evolved the parameters in such structural networks to deliver robust and optimal (for each particular situation) performance in relaying external signals into the cell [4, 5, 6, 7, 8]. In this paper, we show how analysis tools from control engineering may be used to provide a specific example of a cellular system which seems to support both of the above hypotheses.

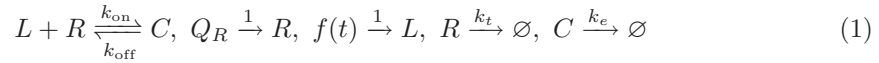
*Dictyostelium discoideum* are social amoebae which have been widely used as model organisms for studying key processes in molecular biology [9]. Under normal conditions, *Dictyostelium* cells grow independently by feeding on bacteria in forest soil, but under conditions of starvation they initiate a well-defined program of development [10]. In this program, the individual cells aggregate by sensing and moving towards gradients in cAMP (cyclic Adenosine Mono-Phosphate), a process known as chemotaxis, to form complexes of up to  $10^5$ -cells. Subsequently, the individual cells form a slug which eventually becomes a fruiting body which emits spores. The early stages of aggregation are initiated by the production of spontaneous oscillations in the concentration of cAMP (and several other molecular species) inside the cell. While the cells are aggregating, they show remarkable sensitivity to small changes in external cAMP concentrations, with only a difference of a few molecules being sufficient to make the cell move correctly towards the region of higher concentration. On the other hand, as *Dictyostelium* cells approach close to each other and form a slug, cell-to-cell adhesion and surface contacts also contribute important effects [11].

In [10, 12] a model, consisting of a set of nonlinear ordinary differential equations, was developed to explain the processes underlying the spontaneous oscillations which occur in the early stages of *Dictyostelium* aggregation. Note that the oscillations for each individual cell are not completely autonomous, but are excited by changes in the concentration of external cAMP, which is secreted from each cell and diffuses throughout the region where the cells are distributed. Thus, for this system, external cAMP molecules constitute the ligand, while molecules on the surface of the *Dictyostelium* cells called CAR1 (Catabolism of ARGinine) constitute the receptors.

In this paper, we show that the above ligand/receptor interaction network exhibits the generic network structure postulated in [3]. The dynamics of the *Dictyostelium* cAMP network for both low and high external cAMP concentrations are compared with the responses of the epidermal growth factor receptor (EGFR) network, which is related to development and tumorigenesis, the transferrin receptor (TfR) network, which enables iron uptake from the extracellular space, and the vitellogenin receptor (VtgR) network, which is a transport receptor used during oogenesis in many oviparous species [3].

## 2. A Generic Structure for Ligand/Receptor Interaction Networks

A generic structure for cellular ligand/receptor interaction networks of the following form is proposed in [3]:



where  $L$  is the ligand concentration,  $R$  is the number of external cell receptor molecules,  $C$  is the number of ligand-receptor complex molecules,  $k_{\text{on}}$  is the forward reaction rate for ligands binding to receptors,  $k_{\text{off}}$  is the reverse reaction rate for ligands dissociating from receptors,  $k_t$  is the rate of internalisation of receptor molecules,  $k_e$  is the rate of internalisation of ligand-receptor complexes, and  $Q_R$  is equal to  $R_T \times k_t$ .  $R_T$  is the steady state number of cell surface receptors when  $C = 0$  and  $L = 0$ ,  $\emptyset$  represents the sinks of either the receptor or the complex,  $f(t)$  is an external stimulus signal and  $t$  is time. The corresponding differential equations are given by

$$\frac{d}{dt} \begin{bmatrix} R \\ C \\ L \end{bmatrix} = \begin{bmatrix} -k_{\text{on}}RL + k_{\text{off}}C - k_tR + Q_R \\ k_{\text{on}}RL - k_{\text{off}}C - k_eC \\ -\frac{k_{\text{on}}}{N_{av}V_c}RL + \frac{k_{\text{off}}}{N_{av}V_c}C + f(t) \end{bmatrix} \quad (2)$$

where  $N_{av}$  is Avogadro's number,  $6.023 \times 10^{23}$  and  $V_c$  is the cell volume in liters throughout which the receptors are distributed.

In normalised form, the above equation can be written as

$$\frac{d}{dt^*} \begin{bmatrix} R^* \\ C^* \\ L^* \end{bmatrix} = \begin{bmatrix} -R^*L^* + C^* - \alpha(R^* - 1) \\ R^*L^* - C^* - \beta C^* \\ -\gamma R^*L^* + \gamma C^* + u(t) \end{bmatrix} \quad (3)$$

where  $t^* = k_{\text{off}} t$ ,  $R^* = R/R_T$ ,  $C^* = C/R_T$ ,  $L^* = L/K_D$ ,  $u(t) = f(t)/(k_{\text{off}} K_D)$   $K_D$  is the receptor dissociation constant, i.e.,  $K_D = k_{\text{off}}/k_{\text{on}}$ ,  $\alpha = k_t/k_{\text{off}}$ ,  $\beta = k_e/k_{\text{off}}$ ,  $\gamma = K_a R_T/(N_{av}V_c)$  and  $K_a = 1/K_D$ .  $\alpha$  is a quantity proportional to the probability of internalisation of unbound receptors,  $\beta$  is a quantity proportional to the probability of internalisation of captured ligand by receptors before dissociation of the ligand from the receptors, and  $\gamma$  represents the level of sensitivity of the receptors to the external signals [3].

The above kinetics can be simplified for two extreme cases, i.e., low and high ligand concentrations. These two cases are of particular interests for various biomolecular networks and they demonstrate different optimality and robustness properties of the ligand-receptor

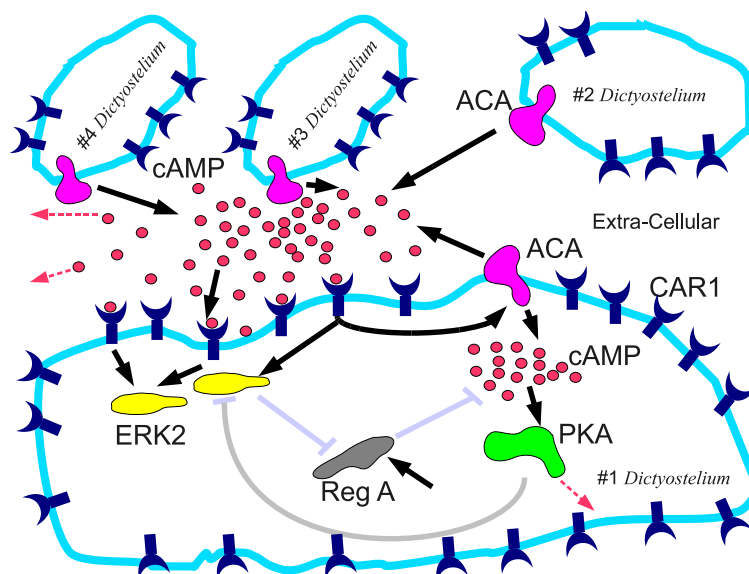


Figure 1. *Dictyostelium* cAMP Oscillation Network, where the figures are not to scale and the details for #2, #3 and #4 *Dictyostelium*'s are omitted.

kinetics. By assuming that the number of receptors is much larger than the number of ligands, i.e.  $dR/dt \approx 0$  ( $R \approx R_T$ ), which is the case for low ligand concentrations, the following approximations for the ligand/complex and ligand kinetics are obtained:

$$\frac{d}{dt^*} \begin{bmatrix} C^* \\ L^* \end{bmatrix} = \begin{bmatrix} -(1 + \beta) & 1 \\ \gamma & -\gamma \end{bmatrix} \begin{bmatrix} C^* \\ L^* \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} u(t) \quad (4)$$

On the other hand, by assuming that the number of ligands is much larger than the number of receptors, i.e.  $dL/dt \approx 0$  ( $L \approx \text{constant}$ ), which is the case for high ligand concentrations, the following approximations for the ligand/complex and receptor kinetics are obtained:

$$\frac{d}{dt^*} \begin{bmatrix} C^* \\ R^* \end{bmatrix} = \begin{bmatrix} -(1 + \beta) & L^* \\ 1 & -(L^* + \alpha) \end{bmatrix} \begin{bmatrix} C^* \\ R^* \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} \alpha \quad (5)$$

where  $L^*$  remains approximately constant and is equal to  $L/K_D$ .

### 3. Ligand/Receptor Interaction Network of Aggregating *Dictyostelium* Cells

We now show how a ligand/receptor interaction network displaying the generic structure given in the previous section may be extracted in a straightforward manner from a model for the network underlying cAMP oscillations in *Dictyostelium* published in [10, 12] and extended for synchronising mechanism in [13], and shown in Figure 1. In the figure, arrows show activation, broken arrows show degradation and the bar arrows represent inhibition.

The corresponding model consists of a set of nonlinear differential equations in the following

form:

$$\begin{aligned}
\frac{d[ACA]}{dt} &= k_1[CAR1] - k_2[ACA][PKA] \\
\frac{d[PKA]}{dt} &= k_3[cAMPi] - k_4[PKA] \\
\frac{d[ERK2]}{dt} &= k_5[CAR1] - k_6[PKA][ERK2] \\
\frac{d[RegA]}{dt} &= k_7 - k_8[ERK2][RegA] \\
\frac{d[cAMPi]}{dt} &= k_9[ACA] - k_{10}[RegA][cAMPi] \\
\frac{d[cAMPe]}{dt} &= k_{11}[ACA] - k_{12}[cAMPe] + \sum_{i=2}^n \tilde{k}_{11}^i [ACA_i] \\
\frac{d[CAR1]}{dt} &= k_{13}[cAMPe] - k_{14}[CAR1]
\end{aligned} \tag{6}$$

where ACA is adenylyl cyclase, PKA is the protein kinase, ERK2 is the mitogen activated protein kinase, RegA is the cAMP phosphodiesterase, and cAMPi and cAMPe are the internal and the external cAMP concentrations, respectively. CAR1 stands for the ligand-bound cell receptor but in the above equation it indicates the ligand-receptor complex.  $\sum \tilde{k}_{11}^i [ACA_i]$  is the contribution to the external cAMP from the other *Dictyostelium*, where the diffusion effect is negligible as the distance between cells are assumed to be close enough,  $n$  is the number of cells and  $\tilde{k}_{11}^i$  is the external cAMP secretion rate for the  $i$ -th *Dictyostelium*, which is not necessarily the same value as the ones for the other *Dictyostelium*.

In the next two sections, the biologically important cases of low and high ligand concentrations are considered. From now on,  $L$  and  $C$  correspond to cAMPe and CAR1 in (6), respectively, and the same notation  $R$  is used for the CAR1 receptor.

### 3.1. Low Ligand Concentration

Consider first the low ligand concentration case. The ligand-receptor interaction network for this case can be extracted from (6) as follows:

$$\frac{d}{dt} \begin{bmatrix} [CAR1] \\ [cAMPe] \end{bmatrix} = \begin{bmatrix} -k_{14} & k_{13} \\ 0 & -k_{12} \end{bmatrix} \begin{bmatrix} [CAR1(t)] \\ [cAMPe(t)] \end{bmatrix} + \begin{bmatrix} 0 \\ k_{11} \end{bmatrix} [ACA(t)] + \sum_{i=2}^n \begin{bmatrix} 0 \\ \tilde{k}_{11}^i \end{bmatrix} [ACA_i(t)] \tag{7}$$

More than 10 cells are locally synchronised in the aggregation phase [14], the effect of  $[ACA]$  could be ignored as comparing with the ones from the other cells as follows:

$$\frac{d}{dt} \begin{bmatrix} [CAR1] \\ [cAMPe] \end{bmatrix} \approx \begin{bmatrix} -k_{14} & k_{13} \\ 0 & -k_{12} \end{bmatrix} \begin{bmatrix} [CAR1(t)] \\ [cAMPe(t)] \end{bmatrix} + \sum_{i=2}^n \begin{bmatrix} 0 \\ \tilde{k}_{11}^i \end{bmatrix} [ACA_i(t)] \tag{8}$$

Note that in the above,  $[CAR1(t)]$ ,  $[cAMPe(t)]$ ,  $[ACA(t)]$  and  $[ACA_i(t)]$  are concentrations in units of  $\mu\text{M}$ , and  $k_{11}$ ,  $\tilde{k}_{11}^i$ ,  $k_{12}$ ,  $k_{13}$  and  $k_{14}$  are reaction constants in units of 1/min. To transform the unit of  $CAR1(t)$  into the number of molecules, we use the relation,  $C = [CAR1(t)]N_{av}V_c$ , and hence derive the following:

$$\frac{dC}{dt} = -k_{14}[CAR1(t)]N_{av}V_c + k_{13}[cAMPe(t)]N_{av}V_c = -k_{14}C + k_{13}N_{av}V_cL \tag{9}$$

where  $L = [cAMPe(t)]$ . In addition,

$$\frac{dL}{dt} = -k_{12}L + \sum_{i=2}^n \tilde{k}_{11}^i [ACA_i(t)] \quad (10)$$

With the normalised states,

$$\frac{dC^*}{dt^*} = -\frac{k_{14}}{k_{\text{off}}}C^* + \frac{k_{13}N_{av}V_c}{R_T k_{\text{on}}}L^* \quad (11)$$

Then,

$$\frac{dC^*}{dt^*} = -\frac{k_{14}}{k_{\text{off}}}C^* + L^{**} \quad (12)$$

where  $L_L^{**} = L^*K_L$  and  $K_L = (k_{13}N_{av}V_c)/(R_T k_{\text{on}})$ . Note that  $K_L$  is multiplied by  $L^*$  to make the coefficient equal to one as in (4). Similarly,

$$\frac{dL_L^{**}}{dt^*} = -\frac{k_{12}}{k_{\text{off}}}L_L^{**} + u \quad (13)$$

where

$$u = \frac{K_L}{K_D k_{\text{off}}} \sum_{i=2}^n \tilde{k}_{11}^i [ACA_i(t)] \quad (14)$$

In a compact form,

$$\frac{d}{dt} \begin{bmatrix} C^* \\ L_L^{**} \end{bmatrix} = \begin{bmatrix} -k_{14}/k_{\text{off}} & 1 \\ 0 & -k_{12}/k_{\text{off}} \end{bmatrix} \begin{bmatrix} C^* \\ L_L^{**} \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} u \quad (15)$$

Comparing (15) with (4), we notice that there are some differences in the structures of the two equations. However, this is mainly because of the effect of the  $k_{\text{off}}C$  term in (2). Including only the external response part, (4) can be rewritten as follows:

$$\frac{d}{dt^*} \begin{bmatrix} C^* \\ L_L^{**} \end{bmatrix} = \begin{bmatrix} -\beta & 1 \\ 0 & -\gamma \end{bmatrix} \begin{bmatrix} C^* \\ L_L^{**} \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} u \quad (16)$$

Then, the following relations are obtained:

$$\beta = \frac{k_{14}}{k_{\text{off}}}, \quad \gamma = \frac{k_{12}}{k_{\text{off}}}, \quad (17)$$

Although the generic ligand-receptor interaction network structure certainly seems to be used in this model of how *Dictyostelium* cells generate cAMP oscillations, it can be immediately seen that a profound difference also exists. Unlike (12), the effect of  $C^*$  to  $dL_L^{**}/dt^*$  is zero. Thus, the rate of dissociation of the ligand from the receptor is very low, i.e. once the cAMP ligand is caught by the CAR1 receptors, it is rarely released before being absorbed into the cell.

The values of the constants in the above equations are given as follows:  $k_{11} = 0.7 \text{ min}^{-1}$ ,  $k_{12} = 4.9 \text{ min}^{-1}$ ,  $k_{13} = 23.0 \text{ min}^{-1}$ ,  $k_{14} = 4.5 \text{ min}^{-1}$ ,  $R_T = 4 \times 10^4$ , [12, 15], and  $k_{\text{off}} = 0.7 \times 60 \text{ min}^{-1}$  and  $k_{\text{on}} = 0.7 \times 60 \times 10^7 \text{ M}^{-1} \text{ min}^{-1}$  [16]. Hence,  $\beta = 0.107$  and  $\gamma = 0.117$ . In [17], the average diameter and volume of a *Dictyostelium* cell are given by  $10.25 \text{ }\mu\text{m}$  and  $565 \text{ }\mu\text{m}^3$ . To

calculate  $V_c$ , we consider an approximation for the shape of a *Dictyostelium* cell as a cylinder. Since the cell receptors are only distributed on the surface of the cell, the interior of the cell must be extracted to calculate an effective volume that represents the space where all molecular interactions occur under well-mixed conditions. The effective volume is determined such that the maximum number of ligand-bound CAR1 molecules is about 1% of the total number of receptors, to give a value of  $V_c$  equal to  $1.66 \times 10^{-16}$  liters. These values were verified using a stochastic simulation of the Laub-Loomis model with Gillespie's direct method [18].

### 3.2. High Ligand Concentration

Similarly, the ligand-receptor interaction network for high ligand concentrations can be extracted from (6) as follows:

$$\frac{d}{dt} \begin{bmatrix} C \\ R \end{bmatrix} = \begin{bmatrix} -k_{14}C + k_{13}N_{av}V_c[\text{cAMPe}(t)] \\ -k_{13}N_{av}V_c[\text{cAMPe}(t)] - k_t R + Q_R \end{bmatrix} \quad (18)$$

where  $k_{13}$  corresponds to the rate of ligand-receptor complex generation where the number of receptors is constant. Therefore, in this case the number of unoccupied receptors is changing significantly and thus  $k_{13}N_{av}V_c$  is replaced by  $k_{on}R$ . Hence,

$$\frac{d}{dt} \begin{bmatrix} C \\ R \end{bmatrix} = \begin{bmatrix} -k_{14} & k_{on}L \\ 0 & -k_{on}L - k_t \end{bmatrix} \begin{bmatrix} C \\ R \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} Q_R \quad (19)$$

where for this case of high ligand concentrations the concentration of cAMPe, i.e.  $L$ , is considered as a constant,  $C$  and  $R$  are in units of the number of molecules and  $L$  is in units of M (Molar). The ligand-receptor complex is assumed to be completely internalised as in the low ligand concentration case. Normalising in the same way as for the previous case, the following equations are obtained:

$$\frac{d}{dt^*} \begin{bmatrix} C^* \\ R^* \end{bmatrix} = \begin{bmatrix} -\beta & L_a^{**} \\ 0 & -(L_a^{**} + \alpha) \end{bmatrix} \begin{bmatrix} C^* \\ R^* \end{bmatrix} + \begin{bmatrix} 0 \\ 1 \end{bmatrix} \alpha \quad (20)$$

where  $L_a^{**} = K_a L$ . Again, the rate of dissociation of the ligand from the receptor is assumed to be very low and the effect of  $C^*$  to  $dR^*/dt^*$  is zero.

## 4. Dynamic characteristics of the *Dictyostelium* ligand/receptor interaction network

In this section we analyse the optimality and robustness of the parameters in the *Dictyostelium* ligand/receptor interaction network for low and high ligand concentrations.

### 4.1. Low Ligand Concentration

For the low ligand concentration case, differentiating both sides of (12) with respect to the normalised time,  $t^*$ , we get

$$\frac{d^2 C^*}{dt^{*2}} = \frac{-k_{14}}{k_{off}} \frac{dC^*}{dt^*} + \frac{dL^{**}}{dt^*} = \frac{-k_{14}}{k_{off}} \frac{dC^*}{dt^*} - \frac{k_{12}}{k_{off}} \left( \frac{dC^*}{dt^*} + \frac{k_{14}}{k_{off}} C^* \right) + u \quad (21)$$

Hence, the ligand/receptor complex kinetics for the low ligand concentration case are given by

$$\ddot{C}^* + \frac{k_{12} + k_{14}}{k_{off}} \dot{C}^* + \frac{k_{12}k_{14}}{k_{off}^2} C^* = u \quad (22)$$



Table I. Kinetic parameters for EGFR, Tfr and VtgR ligand-receptor networks [3]

	$k_e$	$k_t$	$k_{\text{off}}$	$K_D$ [nM]	$R_T$	$V_c$
EGFR	0.15	0.02	0.24	2.47	$2 \times 10^5$	$4 \times 10^{-10}$
Tfr	0.6	0.6	0.09	29.8	$2.6 \times 10^4$	$4 \times 10^{-10}$
VtgR	0.108	0.108	0.07	1300	$2 \times 10^{11}$	$4 \times 10^{-10}$

where the single and the double dot represent  $d(\cdot)/dt^*$  and  $d^2(\cdot)/dt^{*2}$ , respectively.

Since (22) is simply a second-order linear ordinary differential equations, we can define the natural frequency,  $\omega_n$ , and the damping ratio,  $\zeta$  as follows:

$$\ddot{C}^* + 2\zeta\omega_n\dot{C}^* + \omega_n^2 C^* = u \quad (23)$$

Comparing (22) with (23) we have that

$$\omega_n = \frac{\sqrt{k_{12}k_{14}}}{k_{\text{off}}}, \quad \zeta = \frac{k_{12} + k_{14}}{2\sqrt{k_{12}k_{14}}} \quad (24)$$

Substituting the appropriate values for the *Dictyostelium* network, we find that  $\omega_n$  is equal to 0.112 and  $\zeta$  is equal to 1.001. The overshoot,  $M_p$ , and the settling time,  $t_s$ , for a step input are given by [19]

$$M_p = \begin{cases} e^{-\pi\zeta\sqrt{1-\zeta^2}}, & \text{for } 0 \leq \zeta < 1 \\ 0, & \text{for } \zeta \geq 1 \end{cases} \quad (25a)$$

$$t_s = \frac{-\ln 0.01}{\zeta\omega_n} \quad (25b)$$

Thus, the kinetics of the *Dictyostelium* ligand/receptor network produce a system with a damping ratio almost exactly equal to 1, i.e. the critical damping ratio. The critical damping ratio is the optimal solution for maximising the speed of a system's response without allowing any overshoot:

$$\zeta^* = \arg \min t_s(\zeta) \quad (26)$$

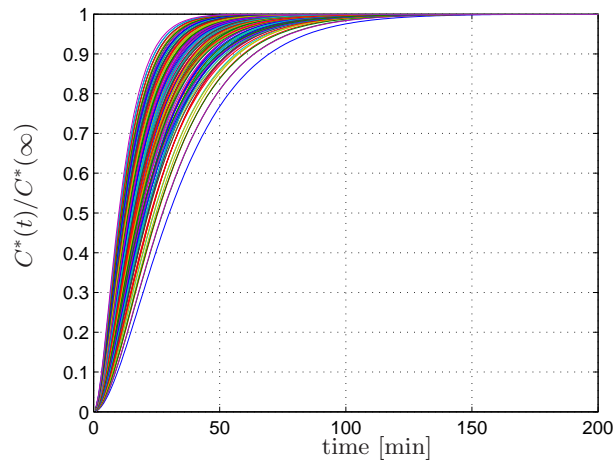
subject to  $M_p = 0$  and (22). It appears that *Dictyostelium* cells may have evolved a receptor/ligand interaction network which provides an optimal trade-off between maximising the speed of response and prohibiting overshoot so that it can follow oscillatory external signals with a certain frequency.

In Table I, the values of the network parameters for three other ligand-receptor networks (discussed in [3]) are shown. As is clear from the values of the damping ratio of *Dictyostelium* for low and high ligand concentration cases (see Table II), the responses are over-damped and thus no overshoot to fast changes in ligand concentrations will occur. Indeed, in the case of the *Dictyostelium* network, the possibility of overshoot is completely prohibited, since the damping ratio cannot be less than one for any combination of the kinetic parameters. This can be seen by considering the fact that

$$\zeta = \frac{k_{12} + k_{14}}{2\sqrt{k_{12}k_{14}}} \geq 1 \Leftrightarrow (k_{12} - k_{14})^2 \geq 0 \quad (27)$$

Table II. Damping ratio ( $\zeta$ ) and natural frequency ( $\omega_n$ ) for low and high ligand concentration

	$L \ll 1\mu\text{M}$		$L = 10\mu\text{M}$	
	$\zeta$	$\omega_n$	$\zeta$	$\omega_n$
EGFR	1.40	0.58	0.18	20.12
TfR	63.70	0.06	1.59	5.79
VtgR	0.05	25.27	1.36	0.88
<i>Dictyostelium</i>	1.00	0.11	15.3	3.27

Figure 2. Step responses with the perturbed parameters  $k_{12}$  and  $k_{14}$ .

for all  $k_{12} > 0$  and  $k_{14} > 0$ . Hence, the over-damped nature of the dynamical response stems from the network structure itself, rather than being dependent on any particular value of the kinetic parameters. For this level of uncertainty in the kinetic parameters, the settling times vary between 35 min and 105 min (for the nominal parameter values the settling time is about 52 min). Each step response for various perturbation combinations is shown in Figure 2, where each kinetic parameter is perturbed by up to  $\pm 50\%$  and the response is normalised by the value of each steady state.

One significant difference between the *Dictyostelium* network and the other ligand-receptor networks shown in Table I is its relatively fast response time. Since aggregating *Dictyostelium* cells exhibit oscillatory behaviour, rather than converging to a constant steady-state, the ligand/receptor interaction network may have evolved to maximise the speed of response, in order to ensure the generation of robust and stable limit cycles for low concentrations of external cAMP. This can be more clearly seen in the Bode plots for the responses of the different networks, which are shown in Figure 3(a).

The region inside the two dashed vertical lines corresponds to oscillations with periods between 5 and 10 minutes, which is the range of cAMP oscillations observed experimentally in

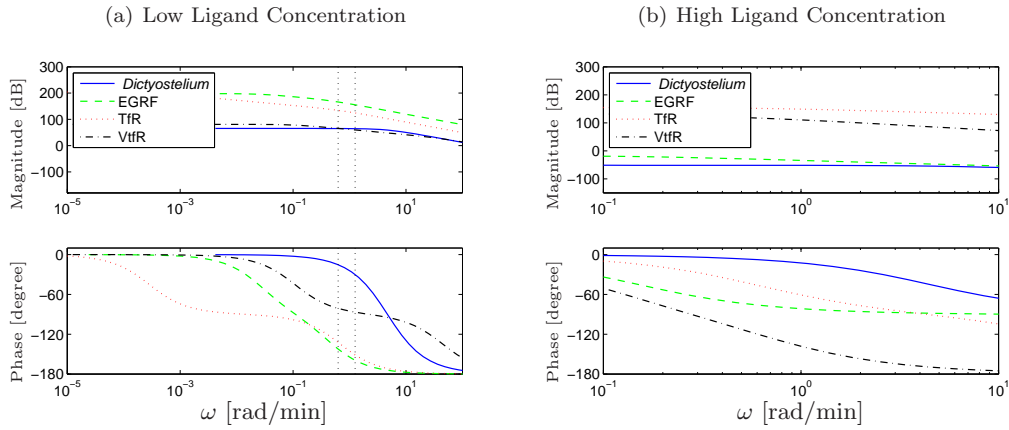


Figure 3. Bode Plots for Low/High Ligand Concentrations

the early stages of aggregation of *Dictyostelium*. The bandwidth of the *Dictyostelium* ligand-receptor kinetics is about 3 rad/min, which is just above the minimum necessary to facilitate the oscillations in cAMP with a period of 5 to 10 min observed in *Dictyostelium* during chemotaxis.

Recall that from the definition of  $u(t)$ ,

$$u = \frac{K_L}{K_D k_{\text{off}}} \sum_{i=2}^n \tilde{k}_{11}^i [\text{ACA}_i(t)] = \frac{k_{13} N_{av}}{k_{\text{off}}^2} \frac{V_c}{R_T} \sum_{i=2}^n \tilde{k}_{11}^i [\text{ACA}_i(t)] \quad (28)$$

the cell volume,  $V_c$ , and the total number of receptors,  $R_T$ , appear only in the definition of  $u$  in (22). Hence, variations in  $V_c$  and  $R_T$  can affect the static gain of the response but they have no effect on its dynamic characteristics. Moreover, it is most likely that the total number of receptors increases as the cell volume increases, i.e.  $V_c/R_T \approx (\text{constant})$ . Under this assumption, even the static gain will be relatively insensitive to variations in the cell volume and in the number of receptors. Therefore, the maximum peak ( $M_p$ ) of the network's impulse responses should be very robust with respect to variations in the cell volume ( $V_c$ ) and in the number of cell receptors ( $R_T$ ).

#### 4.2. High Ligand Concentration

Similarly, for the high ligand concentration case, the following is obtained:

$$\ddot{C}^* + \frac{k_t + k_{\text{on}}L + k_{14}}{k_{\text{off}}} \dot{C}^* + \frac{(k_t + k_{\text{on}}L) k_{14}}{k_{\text{off}}^2} C^* = L_a^{**} \alpha \quad (29)$$

Again, comparing (29) with (23), the natural frequency and the damping factor are given by

$$\omega_n = \frac{\sqrt{(k_t + k_{\text{on}}L) k_{14}}}{k_{\text{off}}}, \quad \zeta = \frac{k_t + k_{\text{on}}L + k_{14}}{2\sqrt{(k_t + k_{\text{on}}L) k_{14}}} \quad (30)$$

where  $L$  is assumed to be equal to  $10\mu\text{M}$ , which is about a twenty-times higher concentration than that produced in normal cAMP oscillations during the early stages of *Dictyostelium*

chemotaxis. Substituting the values for the *Dictyostelium* network, we find that  $\omega_n$  is equal to 3.27 and  $\zeta$  is equal to 15.3. As noted in [21],  $k_t$  could vary within a twenty-times range from the minimum to the maximum, i.e. between  $0.012 \text{ min}^{-1}$  and  $0.22 \text{ min}^{-1}$ . However, the value of  $k_t$  is significantly smaller than  $k_{\text{on}}L$  or  $k_{14}$  and thus the effects of  $k_t$  in the natural frequency and the damping factor are negligible. Hence, the ligand/receptor complex kinetics for the high ligand concentration case are approximated by

$$\ddot{C}^* + \frac{k_{\text{on}}L + k_{14}}{k_{\text{off}}} \dot{C}^* + \frac{(k_{\text{on}}L)k_{14}}{k_{\text{off}}^2} C^* = L_a^{**} \alpha \quad (31)$$

Therefore, the dynamics of the network will be highly robust to variations in the receptor internalisation rate, since variations in  $k_t$  only affect the size of the input, i.e.  $\alpha$ , and, considering the high ligand concentration, even this effect is likely to be minor. In addition, similar to the low ligand case the under-damping is prohibited for the high ligand concentration case as well (See Table II).

The Bode plots for the high ligand concentration case are shown in Figure 3(b). The *Dictyostelium* and EGFR networks show the most significant changes in their frequency responses for the two extreme ligand concentration cases. Based on the values of  $\beta$  and  $\gamma$  derived for the *Dictyostelium* and EGFR networks, both may be categorised as dual-sensitivity networks, i.e. these networks achieve an optimal balance between maximising the probability to capture external signals and maximising the probability to internalise the captured signal - see [3] for a full discussion. Finally, as shown in [12], the peak activity of ERK2 during spontaneous cAMP oscillation is when external cAMP concentration is very low concentration, around 1nM. Therefore, the response to high cAMP concentrations would naturally be reduced as characterised in Figure 3. These extreme sensitivity changes in the response of *Dictyostelium* to different external cAMP concentrations may be responsible for the completely different behaviour exhibited by individual *Dictyostelium* cells and the *Dictyostelium* slug formed at the end of the aggregation process.

## 5. Conclusions

Correct functioning of ligand-receptor interactions is crucial to the survival of organisms, and these interactions are mediated by cellular networks whose structure appears to be highly conserved in nature. However, the wide variation in the dynamic responses of different ligand-receptor networks suggests that certain parameters of the network are optimised by evolution to provide appropriate performance and robustness characteristics for particular situations. In this paper, we showed how analysis tools from Control Engineering could be used to study the dynamical characteristics of the ligand-receptor network involved in generating cAMP oscillation in aggregating *Dictyostelium* cells. Using a recently-proposed generic model for ligand-receptor networks, we investigated the system's response in the cases of low and high concentrations of external cAMP, corresponding to two distinct stages of the *Dictyostelium* life cycle. Our analysis revealed highly robust responses for the ligand-bound receptor kinetics for low ligand concentration, and indeed such high levels of robustness are likely to be required from each individual cell in order to survive this stage of its life cycle. *Dictyostelium* cells may have evolved a receptor/ligand interaction network which maximises the speed of response for the given structure that prohibits any overshoot of the response to external signals. On

the other hand, for high ligand concentrations an extreme reduction in the magnitude of the network response to external signals is observed. We postulate that this may be responsible for the completely different physiological behaviour of the organism as groups of up to  $10^5$  *Dictyostelium* cells aggregate to form a slug.

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