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**A Mathematical Modelling Framework for Understanding  
Chemorepulsive Signal Transduction in *Dictyostelium***

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## Abstract

Chemorepulsion is the process by which an organism or a cell moves in the direction of decreasing chemical concentration. While a few experimental studies have been performed, no mathematical models exist for this process. In this paper we have modelled gradient sensing, the first subprocess of chemorepulsion, in *D. discoideum*- a well characterized model eukaryotic system. We take the first steps towards achieving a comprehensive mechanistic understanding of chemorepulsion in this system. We have used, as a basis, the biochemical network of the Keizer-Gunnink et al. (2007) to develop the mathematical modelling framework. This network describes the underlying pathways of chemorepellent gradient sensing in *D. discoideum*. Working with in this modelling framework we address whether the postulated interactions of the pathways and species in this network can lead to a chemorepulsive response. We also analyze the possible role of additional regulatory effects (such as additional receptor regulation of enzymes in this network) and if this is necessary to achieve this behaviour. Thus we have investigated the receptor regulation of important enzymes and feedback effects in the network. This modelling framework generates important insights into and testable predictions regarding the role of key components and feedback loops in regulating chemorepulsive gradient sensing, and what factors might be important for generating a chemorepulsive response; it serves as a first step towards a comprehensive mechanistic understanding of this process.

**Keywords:** Gradient sensing, chemorepellent, spatial signalling, fugetaxis, negative chemotaxis.

## 1. Introduction

Chemotaxis is the locomotive response of a cell or an organism to a chemical concentration gradient. It is observed in the processes of wound healing, tumour metastasis and mammalian development (Eisenbach, 2004). There are two types of chemotaxis- chemoattraction and chemorepulsion. Chemoattraction is the process of migration of cells or organisms upstream of a chemical concentration gradient. In this paper we will focus on chemorepulsion- a type of chemotaxis where cells or organisms migrate away from higher concentrations of a chemical source (Keizer-Gunnink et al., 2007). Synonymous terms for chemorepulsion are 'negative chemotaxis' (Tso and Adler, 1974) and 'fugotaxis' (Vianello et al., 2005). Chemoattraction has been widely studied in a variety of systems through both experiments and mathematical modelling (Skupsky et al., 2005; Iglesias and Devreotes, 2008). Chemorepulsion has been observed in a range of prokaryotic and eukaryotic systems. However, there are fewer experimental studies and no models exist for this process.

An example of chemorepulsive behaviour is seen in neutrophils where these cells migrate away from the chemokine interleukin-8. It has been suggested that chemorepulsion plays an important role in controlling the number of neutrophils at an injury site (Tharp et al., 2006). Chemorepulsion is also observed in *Helicobacter pylori* when these bacteria move away from bile acids and other chemotactic agents in the stomach (Worku et al., 2004). This process is also believed to play an important role in embryo gastrulation (Yang et al., 2002). The aforementioned examples demonstrate the motivation for studying chemorepulsion- in addition to being an interesting phenomenon, it occurs as a part of important physiological processes. In this paper we will focus on the study of chemorepulsion in the eukaryotic system *Dictyostelium*

*discoideum*. This is a well established model system for studying eukaryotic chemotaxis and has been experimentally well characterized. Further, recent experiments have been performed in the context of chemorepulsion, which is the basis of our modelling (see below).

Chemotaxis essentially consists of three subprocesses (Eisenbach, 2004). The first- gradient sensing is the process of sensory transduction by which a cell communicates with its motility machinery after it perceives an external chemical signal. Gradient sensing leads to polarization where signalling elements accumulate at two opposite ends of the cell (Devreotes and Janetopoulos, 2003; Dawes and Edelstein-Keshet, 2007; Krishnan and Iglesias, 2007). This polarization is important for the persistent motion of cells in response to chemical gradients. In the last subprocess, the cell's motility apparatus is activated and it migrates through a well established crawling mechanism (Bruce et al., 2002; Affolter and Weijer, 2005). We will focus on the sensory transduction process as it is the first subprocess of chemorepulsion and it determines the direction in which the cell will move.

The biochemical signalling mechanisms underlying gradient sensing and the other subprocesses in *D. discoideum* are yet to be completely elucidated. Experimental efforts (at the single cell level) have shown that *Dictyostelium* cells exhibit chemoattractive behaviour towards cAMP and chemorepulsive behaviour towards 8CPT-cAMP. Chemical signals are received and mediated by cAR1 and G-Protein subunits; both are present all along the plasma membrane. On reception of the signal, the two enzymes phosphoinositide 3-kinase (PI3K) and PTEN accumulate at the leading edge and rear of the cell, respectively. PI3K phosphorylates phosphatidylinositol (4,5)-bisphosphate, PI(4,5)P<sub>2</sub>, (referred to hence forth as PIP<sub>2</sub>) to form phosphatidylinositol

(3,4,5)-trisphosphate, PI(3,4,5)P<sub>3</sub>, (referred to hence forth as PIP<sub>3</sub>). PTEN mediates the dephosphorylation of PIP<sub>3</sub> to PIP<sub>2</sub>. PI3K is present in a greater concentration at the leading edge while PTEN is present at a greater concentration at the trailing edge. This localized accumulation at opposite ends results in PIP<sub>3</sub> being localized near the leading edge. PIP<sub>3</sub> is involved in mediating the polymerization of actin which leads to the extension of pseudopodia from this side of the cell. This provides the protrusive force that enables the cell to migrate (Keizer-Gunnink et al., 2007). Thus PIP<sub>3</sub> acts as a component of the cell 'compass' (Weiner, 2002; Krishnan and Iglesias, 2004).

In chemoattraction, PLA2 is also involved in directional sensing but its role in chemorepulsion is unknown (Chen et al., 2007; Takeda et al., 2007; Van Haastert et al., 2007). While results have shown that PI3K is not essential for a cell to exhibit chemoattractive migration, it has been found to be necessary for a chemorepulsive response in the experimental studies performed (Keizer-Gunnink et al., 2007). Phospholipase C (PLC) has been observed to be important for a cell to exhibit chemorepulsive behaviour (Keizer-Gunnink et al., 2007). Important experimental work in chemorepulsion has led to the proposal of a single underlying biochemical network for chemorepulsion (and chemoattraction) in *D. discoideum* by Keizer-Gunnink et al. (2007), based on the players which have been shown to be important in this process. This network contains three regulatory pathways (see Fig.1(A) for the elements of the network). The first pathway is where the inhibition of PLC occurs- 8CPT-cAMP is sensed via cAR1 and the signal is directed through GCPR proteins to inhibit PLC. The second regulatory effect involves PTEN binding to PIP<sub>2</sub>. The third is a feedback effect between PI3K and PIP<sub>3</sub> involving F-actin (Keizer-Gunnink et al., 2007). These results are postulated to lead to the accumulation of PIP<sub>3</sub> at the side of the cell where

chemorepulsive signal concentration is the lowest. A very important component of the network is the inhibition or activation of PLC by the chemorepellent or chemoattractant signal respectively (note that henceforth the network of Keizer-Gunnink et al. (2007) will be referred to as the network). PLC is postulated to act as a polarity switch (i.e. the different receptor regulation of PLC- up or downregulation, holds the key to chemoattractive or chemorepulsive behaviour, respectively) (Keizer-Gunnink et al., 2007). Our goal in this paper will be to focus on modelling the chemorepulsive signal transduction.

While this network is based on biochemical entities shown to be important in this process, it is not obvious if the postulated network interactions result in chemorepulsive responses when stimulated by a chemorepellent. Furthermore, the effects of the kinetics and transport of the biochemical components and reactions in the signalling pathways have not been quantitatively studied. In such a network, there are many parameters which are unknown, and further there are possibly some missing regulatory interactions which could play an important role. Now, in the absence of a mathematical modelling framework, it is difficult to systematically assess if the postulated network could result in the desired behaviour. If the network does indeed result in the desired behaviour it is difficult to discern which crucial elements help achieve this behaviour. A mathematical modelling study of the process can tackle these issues and provide insights by expanding the network into a framework, within which other additional regulatory effects can also be systematically investigated. It also serves as a basis for future expansion and refinement of our understanding of the process.

In this paper, we will take the first steps towards obtaining a quantitative, mechanistic understanding of the biochemical signal transduction underlying

chemorepellent gradient sensing in *D. discoideum*. We will develop a mathematical modelling framework based on the network. We will take the basic structure of the network- the biochemical species and their interactions and expand this into a modelling framework to explore the different ways in which the signal is transmitted through these interactions. Different scenarios for signal transduction, one of which is the network exactly as depicted by Keizer-Gunnink et al. (2007), will be examined. Additional regulatory effects mediated by the chemorepellent signal to biochemical elements in this network will be explored. Thus the framework will provide a platform for analyzing different possible ways in which the chemorepellent signal is transmitted through the biochemical signalling pathways and whether the desired behaviour can be achieved. We believe that such an approach is essential for carefully understanding the roles of different pathways in the complex setting of spatial signal transduction. We will perform a computational analysis of the framework to investigate the following- 1) the quantitative role of PLC in the network (given the fact that this has been part of the focus of experimental work by Keizer-Gunnink et al. (2007)), 2) different forms of receptor signalling to the biochemical components and 3) the roles of the proposed feedbacks. In building this modelling framework we aim to generate a deeper understanding of chemorepulsion and the spatiotemporal signal transduction therein as well as provide testable predictions for further investigations and refinement.

## **2. Modelling**

In this section, the mathematical modelling framework that we developed is described in detail (Fig. 1). The key biochemical species PLC, DAG, PIP<sub>2</sub>, PIP<sub>3</sub>, PI3K and PTEN and their interactions- 1) the PLC-DAG-PIP<sub>2</sub> pathway, 2) the PI3K-PIP<sub>3</sub>-PIP<sub>2</sub> pathway and 3) the PTEN-PIP<sub>2</sub>-PIP<sub>3</sub> pathway form the basis of this modelling

framework. These pathways and their interactions are studied in detail in the framework. In this framework we first examine the interactions in the network as postulated by Keizer-Gunnink et al. (2007). We also go beyond the signalling pathways in the proposed network to examine possible additional regulatory mechanisms of the enzymes (mainly PI3K and PTEN) by the receptor.

We model the interactions of the species on the membrane of the cell (modelled as a two dimensional disc). This results in a model in a 1-D periodic domain with a length of  $L=2\pi R$  where  $R$  is the radius of the cell.

Our approach to modelling this signalling network is dictated by the need to carefully understand signal transduction in this network, as well as the effects of different pathways and feedback, and how, if at all, they contribute to a chemorepulsive response. In particular, a key challenge is to understand the spatial aspects of signal transduction, which is a significant complication. Thus our approach to modelling is to build a mechanistic spatiotemporal model of reaction kinetics and transport. It is also important to assess what the role of other regulatory mechanisms from the receptor can be and how they work in concert with feedback. This leads naturally to the construction of essentially different variants of models built around the same network, and allows for the careful and systematic assessment of these factors. We believe that working with different variants of models is easier and more transparent than incorporating all effects simultaneously into a single model and studying the different effects by varying parameters.

Representative parameters are chosen, and in this work, the sensitivity of the response to some key parameters/pathways is examined. Further exhaustive sensitivity

analyses will be performed in a future study. In our modelling of various reactions, we will use mass action-kinetics unless otherwise stated. Our approach to modelling is that additional effects (saturation, co-operativity) are incorporated (in the absence of any direct experimental evidence) only if the contributing effect plays a crucial role in the qualitative model behaviour.

## 2.1 PLC-DAG-PIP<sub>2</sub> pathway

PLC is an enzyme which catalyzes the cleavage of PIP<sub>2</sub> into inositol 1,4,5-trisphosphate (Ins(1,4,5)P<sub>3</sub>) and diacylglycerol (DAG). DAG is retained in the membrane while Ins(1,4,5)P<sub>3</sub> diffuses into the cytosol (Gomperts et al., 2003). We only consider DAG in our analysis since we are only modelling the reactions on the membrane. When *D. discoideum* is stimulated with 8CPT-cAMP, which is signalled through the G protein subunit Ga1, PLC activity decreases and does not return to basal levels (Bominaar et al., 1994a; Bominaar et al., 1994b). Therefore we assume that PLC activity is locally (and negatively) regulated by the receptor (implying that PLC activity profile is opposite to that of the chemorepellent). We also assume that under basal conditions the enzyme is present in an active (PLC\*) and inactive form (PLC) which are in equilibrium with one another. The time dependent concentrations of PLC and PLC\* are governed by

$$\partial[PLC]/\partial t = k_{-plc}[PLC^*] + k_s[S][PLC^*] - k_{plc}[PLC] + k_{dplc} \partial^2[PLC]/\partial \theta^2 \quad (1)$$

$$\partial[PLC^*]/\partial t = -k_{-plc}[PLC^*] - k_s[S][PLC^*] + k_{plc}[PLC] + k_{dplc^*} \partial^2[PLC^*]/\partial \theta^2 \quad (2)$$

where  $k_{plc}$  and  $k_{-plc}$  are the kinetic rate constants for the conversion between PLC and PLC\*.  $k_{dplc}$  and  $k_{dplc^*}$  are the diffusion coefficients for PLC and PLC\*, respectively.  $\theta$  is

the position of the species on the membrane of the cell.  $S$  is the chemorepellent signal concentration and  $k_s$  is the kinetic rate constant associated with the receptor mediated conversion of PLC\* to PLC. The equation governing the concentration of DAG is described by

$$\partial[DAG]/\partial t = k_l[PIP_2][PLC^*] - k_{-l}[DAG] + k_{dag} \partial^2[DAG]/\partial \theta^2 \quad (3)$$

where  $k_{-l}$  and  $k_l$  are the reaction rate constants for the conversion between DAG and PIP<sub>2</sub>.  $k_{dag}$  is the diffusion coefficient for DAG. It has been suggested that the regulation of PLC by the receptor is central to the chemorepulsive regulation (Keizer-Gunnink et al., 2007). We will thus pay careful attention to the effect of PLC in our model analysis.

## 2.2 PI3K-PIP<sub>2</sub>-PIP<sub>3</sub> pathway

When the cell is exposed to a chemorepellent gradient, PI3K and its product PIP<sub>3</sub> accumulate at the side of the cell where the concentration of the chemorepellent is the least. PI3K catalyzes the conversion of PIP<sub>2</sub> to PIP<sub>3</sub> which ultimately leads to chemorepulsive movement.

In this paper we investigate two kinds of regulatory effects of PI3K. The first is feedforward regulation by the receptor, and the second is feedback regulation controlled by PIP<sub>3</sub>. As part of our investigations into possible feedforward receptor regulation of PI3K, we will explore two forms of receptor regulation of PI3K in our modelling framework. The first is the **adaptive** receptor regulation of PI3K, which we examine because of the established existence of adaptive signalling in chemoattraction. We model the adaptive regulation of PI3K in the simplest way- involving a combination of local and global pathways (Levchenko and Iglesias, 2002). The second is the **local** regulation of PI3K via the receptor signal. We also examine the hypothesized feedback

from PIP<sub>3</sub> to PI3K (Keizer-Gunnink et al., 2007) to analyze its effect on PI3K regulation and localization.

The adaptive regulation of PI3K is based on the Local Excitation Global Inhibition (LEGI) model of Levchenko & Iglesias in chemoattraction (Levchenko and Iglesias, 2002). In our modelling of the adaptive regulation of PI3K by the receptor signal, we describe the regulation of PI3K localization via a strongly *diffusing* activator enzyme (A) and a *locally acting* inhibitor enzyme (I) (Krishnan, 2009). These enzymes are regulated in a non linear fashion by the receptor signal as in Ma, et al. (2004) (very similar results are seen if this regulation is linear (Alam-Nazki, 2008)). While the identities of the activator and inhibitor are not known we note that this model provides a simple and compact description of adaptive signal transduction to PI3K, which is sufficient for our purposes. Note that we assume that the enzyme PI3K has two forms- an activated binding site associated with this enzyme called PI3K\* and the deactivated or unbound binding site which is denoted by PI3K. The following set of partial differential equations describes the dynamics of A, I and PI3K\* (Krishnan and Iglesias, 2003; Ma, et al., 2004)

$$\partial[A]/\partial t = K_a[S]^2 - K_{-a}[A] + K_{da} \partial^2[A]/\partial \theta^2 \quad (4)$$

$$\partial[I]/\partial t = K_i[S]^2 - K_{-i}[I] \quad (5)$$

$$\partial[PI3K^*]/\partial t = K_f[A][PI3K] - K_r[I][PI3K^*] \quad (6)$$

where  $K_{-a}$ ,  $K_{-i}$ ,  $K_a$ ,  $K_i$ ,  $K_r$ ,  $K_f$  are the kinetic reaction rate constants.  $K_{da}$  is the diffusion coefficient of the activator. We note that this module has the key feature of combining local and global signalling, giving rise to a response, and the non-linearity arises in the

fact that the regulation of the pathways depends on a non-linear function (quadratic) function of the signal. Very similar results are obtained if the regulation depends linearly on the signal.

If PI3K is locally regulated by the chemorepellent signal S then the system of equations is given by

$$d[PI3K^*]/dt = K_f[PI3K] - (K_r[S] + K_c)[PI3K^*] \quad (7)$$

where  $K_c$  is the constant associated with the constitutive degradation of PI3K\* to PI3K. We note that by setting  $K_r$  equal to zero we obtain the case of no receptor regulation of PI3K.

The biochemical network hypothesizes a type of feedback between PI3K and PIP<sub>3</sub> where PI3K localizes at the front due to the production of F-actin via PIP<sub>3</sub> accumulation at the front (Keizer-Gunnink et al., 2007). This can be depicted by the equation

$$d[PI3K^*]/dt = K_f[PI3K] - (K_r[S] + K_c)[PI3K^*] + K_b[PIP_3][PI3K] \quad (8)$$

where  $K_b$  is the kinetic constant for the feedback mechanism. The feedback mechanism describes the effect of increased concentration of active PI3K binding sites, mediated by PIP<sub>3</sub> (other variants of this feedback effect have also been examined (Alam-Nazki, 2008) leading to essentially similar behaviour). While PI3K is responsible for the conversion of PIP<sub>2</sub> to PIP<sub>3</sub>, the reverse of this reaction is catalysed by PTEN.

### 2.3 PIP<sub>2</sub>-PIP<sub>3</sub>-PTEN pathway

We have explored different scenarios for the regulation and localization of PTEN, which broadly fall into two categories: feedforward regulation by the receptor and feedback regulation by its binding to PIP<sub>2</sub>. The feedforward regulation may be either local or adaptive. We note that purely feedforward regulation of PTEN in chemoattraction has been the basis of successfully explaining gradient sensing in immobilized cells (Ma et al., 2004). Correspondingly, feedback regulation is part of the postulated network. We do not combine these effects in our modelling.

We discuss here: 1) Feedforward regulation of PTEN through the receptor via an adaptive mechanism (for a similar but local regulation by receptor see Supporting Material) 2) Regulation of PTEN through feedback effect of PTEN binding to PIP<sub>2</sub>. The model for the adaptive regulation of PTEN is similar to the previously described model for PI3K. However, in this case the activator acts *locally* and the inhibitor is capable of *long range diffusion* (Ma et al., 2004; Krishnan, 2009). The final equations of the model are

$$\partial[A]/\partial t = -K_{-a}[A'] + K_a[S] \quad (9)$$

$$\partial[I']/\partial t = -K_{-i}[I'] + K_i[S] + K_{di} \partial^2[I']/\partial \theta^2 \quad (10)$$

$$\partial[PTEN^*]/\partial t = -K_r[I'][PTEN^*] + K_f[A'][PTEN] \quad (11)$$

where A', I' and PTEN\* are the concentrations of the activator, inhibitor and active bound PTEN, respectively.  $K_{-a}$ ,  $K_{-i}$ ,  $K_a$ ,  $K_i$ ,  $K_r$  and  $K_f$  are kinetic rate parameters for the inactivation and activation reactions.  $K_{di}$  is the diffusion coefficient for the inhibitor.

In the second case we examine an important hypothesis in the network which is the feedback resulting from PTEN binding to PIP<sub>2</sub>. According to the postulation, when a chemorepellent signal inhibits PLC, PIP<sub>2</sub> is hence able to accumulate in the upgradient side of the cell. PTEN binds to PIP<sub>2</sub> and accumulates upgradient as well (Keizer-Gunnink et al., 2007). We have created a mathematical model which captures the essential features of this feedback mechanism as follows. The model contains two pools of PTEN- PTEN on the membrane (PTEN<sub>mem</sub>) and a well mixed cytosolic pool of PTEN (PTEN<sub>cy</sub>), modelled in one spatial dimension for simplicity. PTEN<sub>cy</sub> leaves the cytosolic pool and attaches to the binding site of PIP<sub>2</sub>. Here, PTEN on the membrane is always bound to PIP<sub>2</sub>. Thus, PIP<sub>2</sub> itself (which is only on the membrane) exists in two states- bound to PTEN (PIP<sub>2B</sub>) and not bound to PTEN or unbound (PIP<sub>2UB</sub>). The following equation depicts this relationship

$$[PIP_2] = [PIP_{2UB}] + [PIP_{2B}] \quad (12)$$

The binding of PTEN<sub>cy</sub> to PIP<sub>2UB</sub> can be shown by the following reaction



The amount of PTEN that can leave the cytosol and bind to PIP<sub>2UB</sub> is limited by the amount of available unbound PIP<sub>2</sub>. Therefore

$$[PIP_{2B}] = [PTEN_{mem}] \quad (14)$$

$$[PIP_2] = [PIP_{2UB}] + [PTEN_{mem}] \quad (15)$$

The rate of change of concentration of PTEN<sub>mem</sub> and PTEN<sub>cy</sub> is given by

$$\begin{aligned}
\partial[PTEN_{mem}]/\partial t &= -k_{-ten}[PTEN_{mem}] + k_{ten}[PTEN_{cy}]( [PIP_2] - [PTEN_{mem}] ) + k_{dptenm} \partial^2[PTEN_{mem}]/\partial \theta^2 \\
\partial[PTEN_{cy}]/\partial t &= k_{-ten}[PTEN_{mem}] - k_{ten}[PTEN_{cy}]( [PIP_2] - [PTEN_{mem}] ) + k_{dptenc} \partial^2[PTEN_{cy}]/\partial \theta^2
\end{aligned}
\tag{16,17}$$

where  $k_{-ten}$  and  $k_{ten}$  are the reaction rate constants for the conversion between  $PTEN_{mem}$  and  $PTEN_{cy}$ .  $k_{dptenm}$  and  $k_{dptenc}$  are the diffusion coefficients of  $PTEN_{mem}$  and  $PTEN_{cy}$ , respectively. We have assumed that  $k_{dptenc} \gg k_{dptenm}$ , to reflect the well mixed nature of PTEN in the cytosolic pool (this point is discussed later).

Finally, the partial differential equations for the concentrations of  $PIP_2$  and  $PIP_3$  are given by the following equations:

$$\begin{aligned}
\partial[PIP_2]/\partial t &= k_{re}[PIP_3][PTEN^*] - k_{fo}[PIP_2][PI3K^*] - k_l[PIP_2][PLC^*] \\
&+ k_{-l}[DAG] + k_{dPIP_2} \partial^2[PIP_2]/\partial \theta^2
\end{aligned}
\tag{18}$$

$$\partial[PIP_3]/\partial t = k_{fo}[PIP_2][PI3K^*] - k_{re}[PIP_3][PTEN^*] + k_{dPIP_3} \partial^2[PIP_3]/\partial \theta^2
\tag{19}$$

where  $k_{fo}$  is the forward rate constant and  $k_{re}$  is the backward rate constant of the conversion between  $PIP_2$  and  $PIP_3$ . Note that for the case of PTEN feedback via  $PIP_2$  the term  $PTEN^*$  corresponds to  $PTEN_{mem}$  (from Eq. 16) in Eq. 18 and Eq. 19. Based on different possibilities for receptor regulation and feedback we have created three essentially different model variants. Note that in each variant the local receptor regulation of PLC is a common element. The three model variants are primarily organized according as whether feedback effects are involved in the regulation of PTEN and/or PI3K.

**Model I:** PI3K and PTEN are regulated in a feedforward manner by the chemorepellent receptor signal. The possibility of feedback is not considered in this model.

**Model II:** PI3K is regulated by the chemorepellent receptor signal in a feedforward manner. PTEN is regulated purely by its binding to PIP<sub>2</sub>.

**Model III:** PTEN is regulated by its binding to PIP<sub>2</sub>. PI3K is regulated by a feedback mechanism between PIP<sub>3</sub> and PI3K. The possible role of additional local feedforward regulation of PI3K is also examined.

Note that in Model III removing the local feedforward regulation of PI3K results in the network as depicted in Keizer-Gunnink et al. (2007). Examining these models will tell us whether or not a chemorepulsive response can be achieved in each case and what the contributions of the key pathways and components are therein. The three model variants have been created to achieve a systematic, global analysis of the chemorepulsive signal transduction in *D. discoideum*. Models I to III are transformed into a computational model for numerical analysis. The PDEs are solved in the domain of  $\Theta \in [0, 2\pi]$  with periodic boundary conditions. The PDEs were discretized in space using the finite difference method and simulated in MATLAB, using the stiff solver ode15s. In our simulations we use 100 points to discretize the spatial domain, which resulted in around 1200 ODEs (results were checked by doubling the spatial discretization points). The model response to inputs is simulated and analyzed.

The concentrations of PIP<sub>2</sub>, PIP<sub>3</sub> and DAG are non-dimensionalized by factors representing the total concentration of these lipids at basal conditions. Therefore the dimensionless concentration of the individual species represents the fraction of the total concentration of these species present. This procedure is followed for PLC and PLC\*, PI3K and PI3K\* and PTEN and PTEN\* (and PTEN<sub>mem</sub> and PTEN<sub>cy</sub>) as well. The signal *S* is also non-dimensionalized. Space is made dimensionless by the cell radius which is

of the order of  $5\mu\text{m}$  and time is non-dimensionalized by an appropriate time scale  $\tau$  in the order of seconds. Next the parameters are chosen to reflect the relevant time and spatial scales and basal concentrations and relative amounts of the enzymes and phospholipids present in the cell. The high value of the diffusion coefficient of cytosolic PTEN reflects the well mixed nature of this pool. While the effect of some important parameters is studied here, an exhaustive parametric sensitivity analysis will be done in the future. We note that many of the essential conclusions presented in this paper are largely independent of the particular choice of parameter values (see Supporting Material).

### 3. Results

Our modelling framework explores: 1) The possible receptor signal regulation of PI3K and PTEN, 2) The role of the proposed feedbacks between  $\text{PIP}_2$  and PTEN and  $\text{PIP}_3$  and PI3K and 3) The role of PLC and the postulation that it is the central control of the network. It must be noted that the multiple feedbacks introduce a high level of complexity into the network. Our investigation of the different variants provides insights into these different aspects of signal transduction. We will subject each model variant to a constant external gradient input of strength of 30% (with a maximum at  $\theta=0$ ) and the output we will focus on will be the  $\text{PIP}_3$  concentration profile in particular, which is the key indicator of gradient response in chemorepulsion.

Before we start our investigation of the different models, we note that by adding the equations for DAG,  $\text{PIP}_2$  and  $\text{PIP}_3$ , and denoting the total concentration of these lipids by  $L_{\text{tot}}$ , we have (assuming that the diffusion coefficients of these lipids are all equal, and have a value  $k_d$ )

$$\partial L_{tot}/\partial t = k_d \partial^2 L_{tot}/\partial \theta^2 \quad (20)$$

This equation is the homogeneous diffusion equation and indicates that the total amount of lipid at any location at steady state is a constant. Further, since at basal conditions the total amount of lipid in dimensionless terms was 1, it follows that  $L_{tot} = 1$ , for all time.

This is valid irrespective of whether enzymes are regulated in a feedforward or feedback manner.

### 3.1 Model I: PI3K and PTEN are regulated by the chemorepellent receptor signal in a purely feedforward manner.

We first examine the situation where there is no regulation of PI3K and PTEN, in which case they are present at basal levels. In this case receptor regulation of the network is only through PLC. Fig. 2 shows that the  $PIP_3$  spatial profile in fact displays a gradation characteristic of a (weak) chemoattractive response, with a maximum coincident with the signal maximum. To understand why this is so, we examine the steady state concentration profile of  $PIP_3$ , in the case where the lipids are weakly diffusing (in which case, the steady state profiles are essentially determined by the kinetics of the network):

$$[PIP_3] = 1 / (1 + (k_{re}[PTEN]/k_{fo}[PI3K])(1 + (k_l[PLC]/k_{-l}))) \quad (21)$$

A look at the above equation reveals that  $PIP_3$  at steady state is a decreasing function of PLC concentration and since PLC is negatively regulated by the receptor, the receptor regulation of PLC has an upregulating effect on  $PIP_3$ . The receptor regulation of PLC tends to increase the flux into the  $PIP_2 - PIP_3$  pathway and hence increase  $PIP_3$ . This indicates that in the absence of any feedback regulation of PTEN

and PI3K, some feedforward effects must be necessary to counteract this role of PLC. This leads us to examine the regulation of PI3K and PTEN. Below, for specificity, we examine the case where this feedforward regulation occurs through adaptive signalling with both PI3K and PTEN regulated by the receptor in an adaptive manner (the case where the regulation is local rather than adaptive is examined in the Supporting Material, and the results are qualitatively similar).

The steady state concentration profiles of the key species are monitored after performing the simulation incorporating this receptor regulation of PI3K and PTEN. The maximum in the profile of PIP<sub>3</sub> is now seen in the position ( $\Theta=\pi$ ) where the concentration of the signal is minimum. This is indicative of a chemorepulsive response (Fig. 3A). This result suggests that under adaptive regulation of PI3K\* and PTEN\* by the receptor, the cell is able to show a persistent chemorepulsive response to a constant external gradient. In this case the chemorepulsive profile is basically imposed by PI3K and PTEN, and in spite of the (negative) receptor regulation of PLC. Next we examined the effect of varying PLC concentration on the network. We varied the rate constant  $k_{plc}$  (which is associated with PLC\* production). As the rate constant was increased, the concentration of PLC\* increased and that of PIP<sub>3</sub> decreased (Fig. 3B). This is because an increase in the concentration of PLC\* leads to a decrease in the concentration of PIP<sub>2</sub>. Since the concentrations of PIP<sub>2</sub> and PIP<sub>3</sub> are essentially in equilibrium (for weakly diffusing phospholipids), the concentration of PIP<sub>3</sub> decreases as well. This further underscores the negative relationship between PLC and PIP<sub>3</sub>.

In summary, Model I has been employed to investigate the role of PLC and the feedforward (adaptive) regulation of the enzymes PI3K and PTEN by the receptor signal in chemorepulsive sensing. It predicts that the cell can demonstrate persistent

chemorepulsive behaviour to a constant external gradient as well as an imperfect adaptive response to a homogenous signal input (Alam-Nazki, 2008). PLC regulates the concentration profile of PIP<sub>3</sub> via the lipid network (catalyzing the conversion reaction of PIP<sub>2</sub> to DAG). The results also indicate that the sole regulation of PLC by the receptor signal does not provide a chemorepulsive response and receptor regulation of PI3K and PTEN is essential in achieving this response. Very similar results are obtained for the case of local regulation of PI3K and PTEN (see Supporting Material). In general in these cases, the chemorepulsive response is in fact established by PI3K and PTEN, and in spite of rather than aided by PLC.

### **3.2 Model II: PI3K is regulated in a feedforward manner by the chemorepellent receptor signal. PTEN is regulated by its binding to PIP<sub>2</sub>.**

The difference between this Model and Model I is that here PTEN is regulated purely by its binding to PIP<sub>2</sub> and not by the receptor signal. PI3K is regulated by the receptor in a feedforward manner. For specificity, we examine the case where PI3K feedforward regulation by the receptor is through adaptive signalling (similar results are obtained if this regulation is local).

When an external gradient signal is applied, Model II predicts a chemorepulsive response for the cell. PI3K\* and PIP<sub>3</sub> display a concentration profile with their maxima at a position coinciding with the minimum of that of the signal indicating a chemorepulsive response. The PIP<sub>2</sub> and PTEN<sub>mem</sub> concentration profiles display a variation similar to that of the signal (Fig. 4). Next we investigate the regulation of PTEN by its binding to PIP<sub>2</sub> and its effect on the network. To investigate this effect we varied the parameter  $k_{ten}$  which is the constant associated with the conversion of PTEN<sub>cy</sub>

to  $PTEN_{mem}$  (this effect can also be examined by varying the total concentration of PTEN). When  $k_{ten}$  was increased, the concentration of  $PIP_3$  decreased. The decrease is due to an increase in  $PTEN_{mem}$  concentration which then leads to an increase in the dephosphorylation of  $PIP_3$  on the membrane (Fig. 5).

Finally the role of PLC in regulating the concentration of  $PIP_3$  was evaluated by varying the rate constant  $k_{plc}$ . As  $k_{plc}$  decreased, the concentration of  $PIP_2$  increased (due to a decrease in  $PLC^*$  concentration) (Fig. 6). Interestingly, upon examining the steady state concentration profile of  $PIP_3$  we found that it changed only slightly (relative to  $PIP_2$ ). This result is interesting because of the hypothesised role of the PTEN- $PIP_2$  feedback (Keizer-Gunnink et al., 2007). Also the hypothesized central control of the chemorepulsive network- PLC- seems to have a negligible effect on  $PIP_3$  concentration.

This point can be explained based on an analysis of the steady state concentration profiles of enzymes and lipids. We first note that the total amount of PTEN in the cytosol and the membrane is conserved. This is immediately seen from the equation

$$d/dt \left[ \int_0^{2\pi} [PTEN_{mem}] d\theta + \int_0^{2\pi} [PTEN_{cy}] d\theta \right] = 0 \quad (22)$$

Now examining the expression for PTEN concentration on the membrane at steady state,

$$[PTEN_{mem}] = k_{ten} [PTEN_{cy}] [PIP_2] / (k_{ten} [PTEN_{cy}] + k_{-ten}) \quad (23)$$

we see that it depends on  $PIP_2$  in a nonlinear manner, since the denominator  $PTEN_{cy}$  also depends on  $PIP_2$ , via the conservation condition above. However, since this

cytosolic pool is highly diffusible, the dependence of this quantity on  $PIP_2$  is through a global average only. Thus in terms of the spatial profile of PTEN on the membrane, we see that it is essentially directly proportional to that of the  $PIP_2$  concentration profile.

The other point to be mentioned above is that the cytosolic pool of PTEN in general is expected to have a substantial fraction of the total PTEN. Indeed, in *Dictyostelium* cells, it is estimated that about 75% of the total PTEN is in the cytosol under basal conditions (Iijima and Devreotes, 2002), and this is reflected in our model. Thus it may also be expected that in the above equation,  $PTEN_{cy}$  (which carries hardly any spatial information) is rather weakly affected by PLC modulation. The reason  $PIP_3$  did not vary much with the changing concentration of  $PLC^*$  could be explained via the equilibrium relationship between  $PIP_3$  and  $PIP_2$  shown in Eq. 24 (in the case of non-diffusible or weakly diffusing phospholipids) and this above point.

$$[PIP_3]/[PI3K^*] = C[PIP_2]/[PTEN_{mem}] \quad (24)$$

where the rate constants ( $k_{fo}$  and  $k_{re}$ ) for the interconversion reaction are embedded in the constant  $C$ . The regulation of  $PTEN_{mem}$  via its binding to  $PIP_2$  indicates that the concentration profile of  $PTEN_{mem}$  parallels that of  $PIP_2$  and is essentially proportional, as seen above.

Thus the spatial profile of  $PIP_3$  depends essentially on that of  $PI3K$ . Therefore the ratio of  $PIP_3$  to  $PI3K^*$  in Eq. 24 remains essentially fixed. The individual concentrations of  $PI3K^*$  and  $PIP_3$  do not change because the concentration profile of  $PI3K^*$  only depends on the receptor signal and the mediating enzymes (but not on  $PLC^*$ ). Thus regardless of the changing concentration of  $PLC^*$ , the concentration of  $PI3K^*$  does not vary and  $PIP_3$  concentration remains essentially constant. We conclude

from this result that if the enzyme PI3K is adaptively regulated by the receptor signal and PTEN by its binding to PIP<sub>2</sub>, then the regulation of PLC has a weak effect in regulating the PIP<sub>3</sub> concentration profile. In this case, the concentration profile of PIP<sub>3</sub> is primarily determined by PI3K receptor signal regulation. Note that while for specificity, we have assumed adaptive feedforward regulation of PI3K by the receptor, very similar insights hold good for local feedforward regulation of PI3K.

A further point to be mentioned is that in the above analysis, it was implicitly assumed that the concentration of PIP<sub>2</sub> (and hence PTEN<sub>mem</sub>) was nonzero. In fact there exists a second steady state which is unstable under typical conditions, which corresponds to

$$[DAG] = [PIP_2] = 0, [PIP_3] = 1 \quad (25)$$

This steady state corresponds to no PIP<sub>2</sub> on the membrane, a state which does not correspond to a biologically observed state in this system.

We note that in our modelling, we have treated PTEN in the cytosol as a well mixed pool (reflected in the high diffusion coefficient of cytosolic PTEN). We regard this as an appropriate description. For completeness, we reduced the diffusion of cytosolic PTEN, and this led to some sensitivity of PIP<sub>3</sub> to PLC, when the cytosolic PTEN diffused at a rate comparable to membrane PTEN. However this still resulted in receptor regulation of PLC having a positive effect on PIP<sub>3</sub>, rather than a negative effect.

**3.3 Model III: PTEN is regulated by its binding to PIP<sub>2</sub>. A positive feedback mechanism between PIP<sub>3</sub> and PI3K regulates PI3K. Additional feedforward receptor regulation of PI3K is also examined.**

In this variant we began by investigating if the chemorepellent receptor regulation of PLC alone (along with regulation of PTEN by its binding to PIP<sub>2</sub>) would result in a chemorepulsive response. In order to achieve this we set the rate constant  $K_r$  to zero to remove the effect of signal mediated regulation of PI3K. To start with, the rate constant for feedback between PI3K and PIP<sub>3</sub>,  $K_b$ , was assigned a value of zero as well. The steady state concentration profile of PLC\* showed a variation which was qualitatively opposite to that of the signal. The concentration profiles of PTEN<sub>mem</sub> and PIP<sub>2</sub> were similar to that of the signal. The steady state concentration profiles of PI3K\* and PIP<sub>3</sub> are constant with respect to position (Fig. 7). This is because PI3K\* is not regulated by the signal. Also the ratio of the concentrations of PIP<sub>3</sub> and PI3K\* are proportional to the ratio of the concentrations of PIP<sub>2</sub> and PTEN<sub>mem</sub> (in the case of weakly diffusing lipids) (See Eq. 24). The ratio of PIP<sub>2</sub> to PTEN<sub>mem</sub> displays no spatial variation (due to PTEN binding to PIP<sub>2</sub>) and the concentration of PI3K\* is only dependent on the signal. Thus if receptor regulation of PI3K\* is removed, PI3K\* doesn't exhibit any spatial variation and hence the PIP<sub>3</sub> profile does not vary spatially as well. We conclude that sole regulation of PLC by the receptor and regulation of PTEN via its binding to PIP<sub>2</sub> may not lead to a chemorepulsive response in this Model.

Next, the postulated feedback between PIP<sub>3</sub> and PI3K was explored. This was done by increasing the feedback rate constant  $K_b$ , while keeping other parameters constant ( $K_r$  was still 0 in this case and only PLC was regulated by the receptor signal). The steady state concentration profiles of PIP<sub>3</sub> and PI3K\* indicated that the feedback

rate constant between  $\text{PIP}_3$  and PI3K must be quite large to achieve an increase in the concentration of  $\text{PIP}_3$ . Also, the steady state concentration profiles of PI3K\* and  $\text{PIP}_3$  did not spatially vary (Fig. 8A): the reason for this can be seen in Eq. 24 similar to previous analysis, where a feedback effect between  $\text{PIP}_3$  and PI3K would couple their concentrations in a nonlinear way, but introduce no spatial variation. This result indicates that even with a feedback effect of  $\text{PIP}_3$  on PI3K, if PLC is the only enzyme being regulated by the chemorepellent signal, the cell may not exhibit a chemorepulsive response. Note that this scenario represents the original postulated network. This suggests the need to examine additional receptor regulation of key enzymes to achieve a chemorepulsive response.

We also examined the effect of the postulated feedback between  $\text{PIP}_3$  and PI3K when PLC and PI3K are regulated by the receptor signal. While this result also indicates that a large feedback rate constant is required to obtain an increase in  $\text{PIP}_3$  concentrations, it can be seen that the profile of  $\text{PIP}_3$  does spatially vary and indicates a chemorepulsive response (Fig. 8B). This is due to the receptor regulation of PI3K. Next, we explored the role of PLC in this Model by varying the rate constant  $k_{plc}$  ( $K_b$  was kept constant at a value of 1). As  $k_{plc}$  increased, the concentration of  $\text{PIP}_3$  (and  $\text{PIP}_2$ ) decreased (because the concentration of PLC\* increased) and PI3K\* concentration remained fairly constant (Fig. 9). This result strengthens the case for the weak role of PLC in the localization and regulation of the spatial profile of  $\text{PIP}_3$  in this model (even in the presence of a feedback between PI3K and  $\text{PIP}_3$ ).

In Model III we have evaluated the role of the postulated feedbacks of  $\text{PIP}_3$ -PI3K and  $\text{PIP}_2$ -PTEN as well as the role of PLC (Keizer-Gunnink et al., 2007). The model predicts a chemorepulsive response when PI3K is locally regulated by the

receptor signal. If PLC is the only enzyme regulated by the receptor signal then a chemorepulsive response may not be achieved (even in the presence of a PIP<sub>3</sub>-PI3K feedback). If PI3K is regulated by the receptor signal then PLC regulation has a minimal effect on PIP<sub>3</sub> concentrations. A strong feedback is needed for a significant increase in PI3K and PIP<sub>3</sub> concentrations. Investigation of this model has revealed that the postulated feedback from PIP<sub>2</sub> to PTEN weakens the postulated role of PLC in the regulation of PIP<sub>3</sub> concentrations. Also, increasing the strength of the PIP<sub>2</sub>-PTEN binding itself, keeping other receptor regulations fixed, has an effect opposite to the one postulated by Keizer-Gunnink et al. (2007).

In summary, we have carried out a detailed analysis of the network in our modelling framework. We have explored additional adaptive and local receptor regulation of the enzymes PI3K and PTEN. We have shown that if there is no receptor regulation of PI3K and/or PTEN, then sole regulation of PLC by the receptor signal cannot by itself result in a response representative of chemorepulsive gradient sensing. Our results indicate that feedforward adaptive (or local) regulation of PI3K and/or PTEN can lead to a chemorepulsive response. Interestingly when PI3K is regulated by an adaptive mechanism (or even a local mechanism) by the receptor signal and PTEN only by its binding to PIP<sub>2</sub>, a change in PLC concentration does not have the effect on PIP<sub>3</sub> concentration profiles as postulated by Keizer-Gunnink et al. (2007). Strengthening the PTEN-PIP<sub>2</sub> binding leads to a decrease in the concentrations of PIP<sub>3</sub> when PI3K is locally regulated by the chemorepellent signal.

### **3.4 Investigation of additional effects in producing chemorepulsive biasing**

The modelling studies performed on the network so far suggest that this network as postulated may not generate chemorepulsive biasing. The analysis reveals two key

stumbling blocks: firstly PLC has an inverse relationship with PIP<sub>3</sub>, loosely speaking, hence receptor downregulation has a positive effect on PLC. Secondly if PTEN is regulated purely by its binding to PIP<sub>2</sub>, then this effect does not contribute to creating a chemorepulsive biasing to overcome the first factor. Therefore it is worth examining if additional elements may play an important role. Based on the analysis above, a natural further investigation involves analyzing whether saturation effects may play a role in the enzymatic regulation of PIP<sub>2</sub>. Thus here we now examine the case that the enzymatic degradation of PIP<sub>2</sub> may indeed operate close to saturation. This is a plausible assumption given the relative abundance of this phospholipid.

In order to do this, we describe the enzymatic regulation of PIP<sub>2</sub> using Michaelis-Menten kinetics. This leads to the following equation governing the concentrations of the PIP<sub>2</sub>

$$\begin{aligned} \partial[PIP_2]/\partial t = & -k_{fo}[PIP_2][PI3K^*]/(k_{m_1} + [PIP_2]) + k_{re}[PIP_3][PTEN^*] \\ & - k_l[PIP_2][PLC]/(k_{m_2} + [PIP_2]) + k_{-l}[DAG] + k_{dPIP_2} \partial^2[PIP_2]/\partial \theta^2 \end{aligned} \quad (26)$$

Similar modifications are made in the equations for DAG and PIP<sub>3</sub>. Before we proceed with our investigation, we briefly note that owing to the PTEN binding to PIP<sub>2</sub>, the above equation possesses a steady state which corresponds to [DAG]=[PIP<sub>2</sub>]=0, [PIP<sub>3</sub>]=1, as before. We will not examine this steady state, and instead focus on the steady state corresponding to non-zero [PIP<sub>2</sub>].

We now investigate whether saturation effects could play an important role. We note that far away from saturation, the degradation reactions for PIP<sub>2</sub> display kinetics which are essentially mass-action kinetics, which was examined carefully above. This naturally leads us to examine the other extreme case.

We first analyze the situation where only the PI3K reaction is operating close to saturation (i.e.  $k_{m1} \ll 1$ ). This implies that the degradation of PIP<sub>2</sub> is essentially a zero order reaction. We examine the steady state PIP<sub>3</sub> profile in the limiting case of weak lipid diffusion. This results in

$$[PIP_3] = k_{fo}[PI3K]/k_{re}[PTEN^*] \quad (27)$$

Eq. 27 reveals a striking difference compared to that earlier (Eq. 24): there is no explicit presence of PIP<sub>2</sub> in the steady state equation for PIP<sub>3</sub>. An analysis of this equation reveals a few points. Firstly, if there is no receptor regulation of PI3K and PTEN (and no feedback effects), there is no spatial variation of PIP<sub>3</sub> and thus no chemorepulsive biasing. Secondly if we incorporate a feedback between PIP<sub>3</sub> and PI3K, this does alter the levels of PIP<sub>3</sub>, but does not introduce any spatial variation. This is simply because the above equation does not have any spatial information.

On the other hand if we include the PTEN regulation purely by its binding to PIP<sub>2</sub>, then we do have spatial variation (Eq. 23). In this case PLC catalyzes the PIP<sub>2</sub> degradation, and so PIP<sub>2</sub> displays a profile opposite to PLC. However from the equation above, PIP<sub>3</sub> displays an inverse relationship with PTEN, whose spatial profile mirrors that of PIP<sub>2</sub> just as explained in previous sections. Thus now PIP<sub>3</sub> displays a spatial variation parallel to that of PLC. Thus a local degradation of PLC does lead to a PIP<sub>3</sub> profile which displays a chemorepulsive biasing (Fig. 10).

We build on this analysis, by now additionally examining whether the PLC degradation of PIP<sub>2</sub> acts close to saturation, making it a zero order reaction (i.e.  $k_{m2} \ll 1$ ). By investigating the above equations in this limit, we find that this increases the

chemorepulsive biasing (Fig. 11). Indeed, in this case, the above equation continues to hold good, but the additional equation which holds is

$$[DAG] = k_i[PLC] / k_{-i} \quad (28)$$

again independent of  $[PIP_2]$ . Now  $[PIP_2] = 1 - [DAG] - [PIP_3]$  with the expressions above and this leads to a nonlinear implicit equation for  $[PIP_2]$  (and hence  $[PTEN]$ ).

The role of a possible  $PIP_3$ -PI3K feedback is to somewhat amplify this spatial biasing effect. However the analysis above indicates that the  $PIP_2$  degradation reaction(s) acting close to saturation, as well as the PTEN binding to  $PIP_2$  are needed to achieve a repulsive biasing. In fact with these two factors, the receptor regulation of PLC is able to exert a negative influence on  $PIP_3$  by increasing the PTEN on the membrane without causing an increase of  $PIP_3$  through the increased  $PIP_2$  and the interconversion of  $PIP_2$  and  $PIP_3$ . Note that if the PI3K- $PIP_3$  feedback is made very large above, that would lead to the situation where all the  $PIP_2$  (and hence PTEN) is removed from the membrane. This situation has not been observed biologically.

#### 4.0 Conclusions

We have taken the first steps towards quantitatively investigating chemorepulsive signalling in *D. discoideum* by developing a modelling framework based on the network of Keizer Gunnink et al. (2007). This network incorporates the players which have been experimentally shown to be important in chemorepulsion. Thus we converted the network diagram and postulated interactions into a mathematical model that described the various reactions and diffusional transport. The mathematical modelling framework was designed around the network and consisted of different

model variants. Each variant examined different possibilities of signal transduction through the pathways by including additional receptor regulation of key enzymes so that the roles of receptor regulation as well as different existing feedback mechanisms could be systematically understood and to see whether they would result in the desired behaviour. The essential difference in variants is the way in which PI3K was regulated (either in a feedforward manner by the receptor, or through feedback from PIP<sub>3</sub>), and also in the way PTEN was regulated (either in a feedforward manner by the receptor or in a feedback manner by its binding to PIP<sub>2</sub>). The analysis of these different model variants together allowed for a better global picture of signal transduction, and enhanced the understanding of the possible roles of different modes of signal processing through particular pathways. This approach is especially useful in understanding signal transduction where non-linearity, feedback and spatial aspects are critical. As is evident from our study, reasoning about the spatial localization of different components of the network just based on a network interaction diagram can be misleading.

In the first model variant, PI3K and PTEN were both regulated by the receptor in a feedforward manner. Model analysis revealed that this could lead to robust chemorepulsive sensing behaviour (as seen from the profile of PIP<sub>3</sub>) and that the role of PLC was to modulate this effect. We also noted in this model that removing receptor regulation of PI3K and PTEN resulted in a situation where receptor regulation (of PLC alone) did not result in a chemorepulsive response, emphasizing that the PLC regulation by itself (without any feedback effects) is not geared towards producing chemorepulsive biasing. In this model variant the feedforward regulation of PI3K and PTEN could have been either adaptive or local (or any combination of the two), and the essential result would be unchanged. In fact it is possible to have feedforward regulation to only one of

these enzymes, leading to chemorepulsive profiles. Thus the prediction of this model variant was that chemorepulsive sensing would result primarily from feedforward regulation of PI3K and(/or) PTEN in a manner opposite to that in chemoattraction with PLC playing a modulatory, but opposing role. In Model II PI3K is again regulated in a feedforward manner by the receptor signal but it differs from Model I in that PTEN is regulated by its binding to PIP<sub>2</sub> (and not directly by the receptor signal). Model II exhibited a chemorepulsive response and we found that PIP<sub>3</sub> was actually quite insensitive to the regulation of the lipid network by PLC. This effect could be directly traced to the nature of PTEN regulation, i.e. by its binding to PIP<sub>2</sub>.

The third model variant differed from the second in the regulation of PI3K via a feedback effect from PIP<sub>3</sub> to PI3K (possibly dependent on the presence of F-actin). This did not result in a chemorepulsive profile. Incorporating an additional feedforward regulation of PI3K led to the result that a chemorepulsive response may be obtained in this case, but also a strong feedback from PIP<sub>3</sub> to PI3K is needed to effect a substantial increase in PIP<sub>3</sub> concentration. We again found that variation in PLC concentrations had a minor effect on the profile of PIP<sub>3</sub> which again could be traced to the binding of PTEN to PIP<sub>2</sub>.

Our analysis and examination of Model III, with the receptor regulation of PI3K deactivated (the scenario which represents the network as postulated in Keizer-Gunnink et al. (2007)), reveals the absence of a chemorepulsive response. This indicates that the model developed based on the original network by itself did not result in a chemorepulsive response.

Based on our analysis of these models (all based on mass-action kinetics) we recognized a crucial element as being the dynamics of  $\text{PIP}_2$ . This led us to examine the possibility that the enzymatic degradation of  $\text{PIP}_2$  may be acting close to saturation. By incorporating this effect, we found that now the modified model based on the network could indicate a chemorepulsive biasing. Analysis of this model reveals that the PI3K based degradation of  $\text{PIP}_2$  acting very close to saturation as being a crucial element, in addition to the PTEN binding to  $\text{PIP}_2$ . This is precisely what allows for the receptor downregulation of PLC to cause a  $\text{PIP}_3$  decrease by increasing  $\text{PIP}_2$  (and hence PTEN) without directly causing any increase of  $\text{PIP}_3$ . Any additional feedforward regulation of PTEN and/or PI3K, or feedback loop could lead to the strengthening of the chemorepulsive biasing.

Taken together our analysis reveals various points. If all the enzymatic reactions (especially those involving  $\text{PIP}_2$ ) were acting far from saturation, then the tendency of the receptor regulation of PLC is to result in a chemoattractive rather than chemorepulsive biasing. This then means that additional receptor regulation of enzymes PI3K and PTEN would be necessary to counteract this effect. The role of PTEN binding to  $\text{PIP}_2$  is generally to desensitize  $\text{PIP}_3$  to PLC, but this does not provide the repulsive biasing. The feedback from  $\text{PIP}_3$  to PI3K in concert with these factors does not provide the biasing on the whole.

The enzymatic degradation of  $\text{PIP}_2$  (especially that involving PI3K) acting close to saturation is a crucial element which may provide some degree of repulsive biasing in the above network. When combined with additional elements including some non-linear distortion in local signal transduction, or additional feedforward receptor regulation, it could explain the chemorepulsive behaviour observed. It is worth pointing out that the

enzymatic degradation of  $\text{PIP}_2$  (via PI3K) acting close to saturation is in fact consistent with other models of PI3K and PTEN regulation in chemoattraction. In fact in this case (as explained in the text)  $\text{PIP}_3$  concentrations at steady state would be essentially proportional to the ratio of PI3K to PTEN concentrations and receptor regulation of PI3K and PTEN could explain chemoattractive gradient sensing in immobilized cells as before (Ma et. al., 2004).

The examination of the model variants has yielded different testable predictions. In particular PI3K plays a very important role in signal transduction and inhibiting its activity significantly diminishes the chemorepulsive response. This is consistent with the experimentally established importance of PI3K in chemorepulsion. The models also make specific predictions regarding the manner in which PLC affects signal transduction. In these variants (consistent with the network in Keizer-Gunnink et al., (2007)), PLC affects the network only by its degradation of  $\text{PIP}_2$ . It is worth pointing out that the insights into the model behaviour were obtained using a combination of analytical and numerical analysis, including some specific sensitivity analysis. The analytical results provide important insights into the different contributing effects, which is confirmed by numerical analysis. Finally, we also mention that the insights from the modelling here are also relevant to migration, via local biasing in pre-polarized cells.

Another point which needs to be made concerns the role of PLC as a polarity switch. Based on the regulation of PLC by chemoattractant and chemorepellent in opposite fashion, it has been suggested that this is the key to the difference in repulsive and attractive response. Based on our analysis here and previous work in chemoattractive modelling, we suggest that this postulation, while attractive in its

simplicity, should be treated with caution: indeed there may be feedforward regulation to other enzymes such as PI3K, which may also be switched in some form (or altered in a quite different or non-opposite manner). Other issues related to the polarity switch will be examined elsewhere, where we will examine issues related to repulsive biasing using qualitatively simplified models.

In conclusion we have taken the first steps towards quantitatively understanding signal transduction underlying chemorepulsive sensing in *D. discoideum*. This involved expanding the network (and its interactions) into a mathematical modelling framework and carefully and systematically analyzing contributions of different pathways to signal transduction via different model variants. The modelling work here could form the basis of further model development and refinement. Such a global approach of model building and analysis plays an important role in understanding the complex non-linear spatial signal transduction characteristic of chemorepulsion and helps in disentangling the effects of the various contributing pathways therein.

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## Appendix A

### A.1. Parameters

Representative parameters are chosen to reflect the relevant time and spatial scales, basal concentrations and relative amounts of enzymes and phospholipids. The non-dimensionalization of different entities is discussed in Section 2. Note that representative total concentration of lipids is roughly  $1000/\mu\text{m}^2$  and representative total enzyme binding site concentrations are roughly  $100/\mu\text{m}^2$  (Ma et al., 2004). Kinetic constants were chosen to reflect representative relative steady state basal concentrations and representative distributions of concentrations of PTEN in the membrane/cytosol. Likewise representative diffusion coefficients were chosen to reflect relative diffusivities of different species on the membrane. For instance the diffusion coefficients of the lipids are of the order of  $1\ \mu\text{m}^2/\text{s}$  (Postma and Van Haastert, 2001). The diffusion coefficient of  $\text{PTEN}_{\text{mem}}$  is chosen to reflect a relatively higher diffusivity.  $\text{PTEN}_{\text{cy}}$  is treated in our modelling as an essentially well-mixed pool. The diffusion coefficient of PLC on the membrane was chosen to be higher than that of the lipids. The effect of all these diffusion coefficients have been carefully examined one at a time, in particular for PLC and also  $\text{PTEN}_{\text{mem}}$  and the lipids: both increasing and decreasing the

diffusion coefficients by a factor of 5 led to minimal changes in steady state results. Similarly preliminary sensitivity analysis of steady state concentrations changes in kinetic parameters has been performed (Alam-Nazki, 2008). It is worth emphasizing that most of the essential insights, analyses and conclusions presented in this paper continue to hold good when these various auxiliary parameters are varied. The sensitivity of the steady state of the network to feedback strengths as well as PLC regulation is studied in the text. It is worth pointing out that the numerical analysis presented in the paper has been complemented and backed up by analytical work.

The dimensionless parameters for Model I, II and III are shown in Tables 1, 2 and 3 respectively.

Table 1

<p><b>Kinetic Parameters</b></p> <p><math>k_s=1.0, k_{plc}=1.0, k_{-plc}=1.0, k_i=1.0, k_{-i}=1.0, k_{fo}=1.0, k_{re}=2.0, K_f=1.0, K_r=4.0, K_a=2.0, K_{-a}=2.0, K_i=1.0, K_{-i}=1.0, K_f=1.0, K_r=2.0, K_a=1.0, K_{-a}=1.0, K_i=2.0, K_{-i}=2.0</math></p> <p><b>Diffusion Coefficients</b></p> <p><math>k_{dPIP2}=0.01, k_{dPIP3}=0.01, k_{dplc}=0.1, k_{dplc^*}=0.1, k_{dag}=0.01, k_{da}=10.0, k_{di}=10.0</math></p>
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Table 2

<p><b>Kinetic Parameters</b></p> <p><math>k_s=1.0, k_{plc}=1.0, k_{-plc}=1.0, k_i=1.0, k_{-i}=1.0, k_{fo}=1.0, K_r=4.0, K_f=1.0, K_a=2.0, K_{-a}=2.0, K_i=1.0, K_{-i}=1.0, k_{ten}=2.0, k_{re}=2.0, k_{-ten}=1.0</math></p>
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**Diffusion Coefficients**

$k_{dplc} = 0.1, k_{dplc^*} = 0.1, k_{dptenc} = 10.0, k_{da} = 10.0, k_{dptenm} = 0.1, k_{dPIP3} = 0.01, k_{dPIP2} = 0.01, k_{dag} = 0.01$

Table 3

**Kinetic Parameters**

$k_s = 1.0, k_{plc} = 1.0, k_{-plc} = 1.0, k_f = 1.0, k_{-f} = 1.0, k_{fo} = 1.0, K_f = 1.0, K_r = 4.0, k_{ten} = 2.0, k_{-ten} = 1.0, K_b = 0.0, K_c = 4.0, k_{re} = 2.0, k_{m1} = 0.01, k_{m2} = 0.1$

**Diffusion Coefficients**

$k_{dplc} = 0.1, k_{dplc^*} = 0.1, k_{dag} = 0.01, k_{dPIP2} = 0.01, k_{dPIP3} = 0.01, k_{dptenc} = 10.0, k_{dptenm} = 0.1$

### Figure Captions

**FIGURE 1 Chemorepulsive Signalling Network.** (A) The proposed biochemical network of chemorepulsion in *D. discoideum* (Keizer-Gunnink et al., 2007) is shown. In this postulation, the 8 CPT-cAMP signal is received via cAR1 and G $\alpha$ 1 resulting in the inhibition of PLC. A feedback regulatory effect of PTEN binding to PIP<sub>2</sub> is indicated. A further feedback regulatory effect between PIP<sub>3</sub> and PI3K involving F-actin is postulated. It is suggested that this network can give rise to chemorepulsive biasing.

**The Modelling Framework.** The proposed and additional interactions are analysed in a mathematical modelling framework. (B) **Model I.** The chemorepellent receptor signal S locally regulates PLC and may additionally regulate the enzymes PI3K and PTEN in a feedforward manner. (C) **Model II.** The chemorepellent receptor signal S locally regulates PLC and may additionally regulate PI3K in a feedforward manner. PTEN is regulated via its binding to PIP<sub>2</sub>. (D) **Model III.** The chemorepellent receptor signal S locally regulates PLC and may additionally regulate PI3K in a feedforward manner. PTEN is regulated via its binding to PIP<sub>2</sub> and a feedback exists between PIP<sub>3</sub> and PI3K. Removing the feedforward regulation of PI3K in this model, results in a scenario which is the same as the postulated network.

**FIGURE 2 Model I: Effect of the receptor signal only regulating PLC (with no regulation of PI3K or PTEN) on the network.** The steady state concentration profiles of the key biochemical species are shown here in response to a 30% signal gradient ( $S=1+0.3\cos\theta$ : the signal has a maximum at 0 and a minimum in the middle of the computational domain. This input is used in all subsequent figures). PIP<sub>3</sub> (*solid line*) follows a weak variation parallel to that of the signal input (with a maximum at 0). In this case a chemorepulsive response is not observed.

**FIGURE 3 (A) Model I:** The steady state concentration profiles of the key biochemical species are shown here in response to the input signal. The maximum of the concentration profile of PIP<sub>3</sub> (*solid line* in the right hand side plot) coincides with the position of the minimum of that of the signal which is indicative of a chemorepulsive response. **(B) Effect of Regulation of PLC.** The steady state concentration profile of PIP<sub>3</sub> is shown as a function of the parameter  $k_{plc}$  ( $k_{plc}= 1.0$  (*solid line*), 2.0 (*dots*) and 4.0 (*solid line with circles*)). PIP<sub>3</sub> concentration decreases as  $k_{plc}$  increases (because the concentration of PLC\* increases).

**FIGURE 4 Model II.** The steady state concentration profiles of the key biochemical species are shown for a 30% gradient signal. The concentration profile of PIP<sub>3</sub> (*solid line* in the right hand side plot) is maximum where that of the signal is minimum. This is indicative of a chemorepulsive response. Note that the concentration profile of PTEN<sub>cy</sub> (*solid line with plus signs* in the right hand side plot) remains relatively flat; it represents a well mixed cytosolic pool of PTEN.

**FIGURE 5 The role of the feedback between PTEN and PIP<sub>2</sub> in the network.** The steady state concentration profile of PIP<sub>3</sub> is shown when the parameter  $k_{ten}$  is varied

( $k_{ten}= 1.0$  (solid line), 2.0 (dots), 4.0 (solid line with circles)). As  $k_{ten}$  increases the concentration of PIP<sub>3</sub> decreases. Note that the repulsive response is determined primarily by PI3K.

**FIGURE 6 Effect of regulating PLC on the network.** The steady state concentration profiles of PI3K\*, PTEN<sub>mem</sub>, PIP<sub>2</sub> and PIP<sub>3</sub> are shown when the parameter  $k_{plc}$  is varied ( $k_{plc}= 1.0$  (solid line), 2.0 (dots) and 4.0 (solid line with circles)). As  $k_{plc}$  varies, the concentration of PIP<sub>3</sub> does not change much relative to PIP<sub>2</sub> (the profiles are very close to one another; see text for rationalization). Note that PI3K\* concentration does not change at all.

**FIGURE 7 Model III.** The steady state concentration profiles of the key biochemical species are shown in response to a 30% gradient. PLC is the sole enzyme being regulated by the receptor signal (receptor regulation of PI3K is switched ‘off’). The concentration profile of PIP<sub>3</sub> does not vary spatially because of the absence of receptor signal regulation of PI3K. This is not indicative of a chemorepulsive response.

**FIGURE 8 The role of the feedback between PIP<sub>3</sub> and PI3K.** (A) **Receptor regulation of PI3K is switched off.** The steady state concentration profile of PIP<sub>3</sub> is shown as a function of the feedback parameter  $K_b$ . PI3K is not regulated by the receptor signal in this case. The profile of PIP<sub>3</sub> does not spatially vary and the concentration of these species increases as  $K_b$  increases. A chemorepulsive response is not observed in this case. (B) **PI3K is regulated by the receptor signal.** The steady state concentration profiles of PIP<sub>3</sub> do spatially vary and increase as  $K_b$  increases. Note that for both (A) and (B)  $K_b= 0.0$  (solid line), 1.0 (dots), 5.0 (solid line with circles), 10.0 (solid line with plus signs) and 50.0 (thick solid line).

**FIGURE 9 Effect of regulating active PLC on the network.** The steady state concentration profiles of PIP<sub>2</sub> and PIP<sub>3</sub> are shown when the parameter  $k_{plc}$  is varied ( $k_{plc}= 1.0$  (*solid line*), 2.0 (*dots*) and 4.0 (*solid line with circles*)). The concentration of PIP<sub>3</sub> increases slightly (the concentration of PI3K\* does not change as it depends only on the input). The regulation of active PLC has a minor effect on the PIP<sub>3</sub> concentration profile.

**FIGURE 10 The effect of saturation of the PI3K-PIP<sub>2</sub> pathway.** The steady state concentration profiles of the key biochemical species are shown for a 30% gradient signal. The concentration profile of PIP<sub>3</sub> (*solid line* in the right hand side plot) is maximum where that of the signal is minimum. This is indicative of a relatively weak chemorepulsive response.

**FIGURE 11 The effect of saturation of the PI3K-PIP<sub>2</sub> and PLC-PIP<sub>2</sub> pathway.** The steady state concentration profiles of the key biochemical species are shown for a 30% gradient signal. The concentration profile of PIP<sub>3</sub> (*solid line* in the right hand side plot) is maximum where that of the signal is minimum. This is indicative of a chemorepulsive response.

**Table Captions****Table 1** Parameters for Model I**Table 2** Parameters for Model II**Table 3** Parameters for Model III

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