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An investigation of design principles underlying repulsive and attractive gradient sensing and their switching

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Many important cellular processes rely on cellular responses to spatially graded signals. This response may be either attractive, indicating a positive bias, or repulsive indicating a negative bias. In this paper we consider cells which exhibit both repulsive and attractive gradient sensing responses and aim to uncover the underlying design principles and features of how the networks are wired which could allow a cell to exhibit both responses. We use a modular approach to examine different configurations which will allow for a cell to exhibit both responses and analyze how this depends on the basic characteristics of gradient sensing and downstream signal propagation. Overall our analysis provides insights into how gradient responses can be switched and the key factors which affect this switching.

1 Introduction

Chemotaxis, the directed migration of cells in response to chemicals, is a fundamental cellular process with manifold applications ranging from wound healing, tumour metastasis to immune system function (Eisenbach, 2004). In these systems the external environment is sensed by the ligand binding to specific receptors on the cell surface. The sensing stage leads to the biasing or guiding of cell motility in appropriate directions. In bacterial cells like *E.coli*, the temporal sensing of the ligand leads to the regulation of the tumbling frequency of the flagellum. In the case of eukaryotic cells, the sensing typically leads to the intermediate step of polarization, the persistent localization of key signalling components to opposite ends of the cell leading to the establishment of an axis, which aids persistent movement.

Chemotaxis is of two types, attractive and repulsive. Chemoattraction has been intensively studied experimentally in bacteria, especially in *E.coli*, and also in eukaryotes such as *Dictyostelium*, neutrophils, fibroblasts to name a few. These studies have focussed not only on the qualitative aspects of migration, but also on the underlying signal transduction processes connecting receptor-ligand binding to motility. Some experimental studies have investigated chemorepulsion in systems such as *E.Coli*, *Dictyostelium*, neutrophils and T-cells. Most of these studies have focussed on demonstrating the fact that cells are capable of chemorepulsion, and are not focussed in detail on the signal transduction (Tharp *et al.*, 2006; Keizer-Gunnink *et al.*, 2007). From the modelling perspective, a series of modelling efforts have been aimed at understanding signal transduction in chemoattraction in *E.coli* as well as eukaryotes (for example see (Tindall *et al.*, 2008; Iglesias & Devreotes, 2008) for surveys of the relevant efforts). A mechanistic modelling study of chemorepulsive sensing in *Dictyostelium* was performed by us in a recent paper (Alam-Nazki & Krishnan, 2010).

While different cells may exhibit chemoattraction or chemorepulsion, our focus will be on cells (primarily eukaryotic cells) which exhibit both chemoattraction and chemorepulsion. There are a number of examples of such cases: *Dictyostelium* (under similar conditions) exhibits chemoattraction to cAMP and chemorepulsion to 8CPT-cAMP (Keizer-Gunnink *et al.*, 2007); growth cones can exhibit chemoattraction and chemorepulsion to the same chemical stimulus depending on other factors present in the external medium, or its internal state (Song *et al.*, 1998; Mueller, 1999); leukocytes can exhibit chemotraction and chemorepulsion to Interleukin-8 depending on the strength of the stimulus(Tharp *et al.*, 2006). In this paper we present a framework to examine the possible design principles and features in the underlying signalling networks which allow them to exhibit both chemoattraction and chemorepulsion.

In all these eukaryotic cells (and bacteria) it is expected that the main difference between chemoattraction and chemorepulsion lies in the sensory transduction stage. In general the chemoattractant and the chemorepellent may be different chemicals, and their receptors can be different. However the mechanism of motility in response to both these chemicals is expected to be the same. This involves the regulation of the actin cytoskeleton, by key proteins in the polarization process such as Rac, Rho and (where applicable) Cdc42. There are many natural questions which arise in trying to understand chemoattractive and chemorepulsive signalling in these systems. Is there a common upstream entity whose regulation acts as the key connection between attractive and repulsive sensing? Is the nature of signalling in these systems local or adaptive? Is an underlying spontaneous symmetry breaking mechanism involved and how does this affect the cell's capacity to exhibit both attractive and repulsive sensing?

Different aspects of attractive and repulsive signal transduction have been discussed in the literature. In particular, experimental studies in *Dictyostelium* in chemorepulsion have led to a network model postulated. In this model, the experimentally observed opposite regulation of the enzyme PLC (a regulator of phosphoinositide lipids) by chemoattractant and chemorepellent respectively is postulated to be the key connection between chemoattraction and chemorepulsion from the perspective of signal transduction. Thus PLC is postulated to be a "polarity switch" in this system (Keizer-Gunnink *et al.*, 2007). The notion of a "polarity switch" has been discussed elsewhere in the literature too (Huttenlocher & Poznansky, 2008). In systems like growth cones and T-cells, the fact that competing pathways are involved in the gradient sensing have been demonstrated experimentally. In this paper, we will focus on primarily these two design aspects, how they could work to give attractive and repulsive migration in eukaryotic systems, and how this might depend on the qualitative aspects of signal transduction. In this manner we aim to develop a framework to examine and elucidate various issues regarding attractive and repulsive sensing and their transition. In particular an implicit focus is on the underlying design principles and network regulation which allow the network controlling attractive migration to be exploited/regulated/modified to give rise to repulsive migration in the same cell. We believe that these are natural issues to examine as a first step towards a detailed mechanistic understanding of attractive and repulsive migration in these systems.

At the outset we recognize that the details of signalling in different cells will be different. This is because of both differences in biochemical network details, as well as basic qualitative differences in the nature of cellular signal processing. In order to address these classes of questions, we will work with representative modules of signalling. These modules, taken from the literature, represent different essential characteristics observed. While these modules naturally do not capture all the biochemical signal

transduction complexity, they provide key insights regarding sensing capabilities. These modules must be seen as being building blocks or representing individual pathways in the overall signal transduction. The detailed biochemical understanding of chemotactic response in these various systems needs the integration of various pathway behaviour, as well as qualitative understanding of the nature of signal processing. In these cases it is vital to obtain insights to the questions above to guide the building of detailed models. The nature of the analysis in this paper is such that it is likely to be useful for a wide range of systems.

Attractive and repulsive migratory behaviour has been observed in different systems as mentioned. While the mechanistic understanding of signal transduction in these systems is far from complete, many important qualitative aspects of the behaviour in chemoattractive signalling have been investigated. This aspect underlies our approach in this paper: we aim to exploit these aspects along with the experimental studies on chemorepulsion to postulate and examine some key questions about how attractive and repulsive signalling occurs, which would provide useful insights as well as testable predictions. We believe that the use of qualitatively simplified models is much more appropriate in this context, as it allows us to focus on some key qualitative aspects and obtain transparent insights, without being distracted by the different mechanistic details and gaps to contend with in individual systems. In the building of detailed mechanistic models, one would have to deal with many unknown details which are somewhat tangential to the issue at hand and in many ways obscure the main points; further, one would have to then check what aspects of the detailed models gave rise to the relevant insights, and if this would still hold good if different biochemical variants were employed. Finally for the issue under consideration, any mechanistic model would necessarily incorporate some phenomenological descriptions. Overall our models incorporate succinctly and transparently certain hypotheses whose consequences can then be understood more easily. The investigations of this paper should be seen as a first step in mechanistically investigating such issues in individual systems using a combination of modelling and focussed experiments.

This paper is organized as follows. In the next section we present the representative modules we employ for our analysis. We then examine different network designs (upstream switch, competing effects) which can give rise to both attractive and repulsive biasing, and examine what the consequence of each possibility is, in light of different signal transduction scenarios. We then conclude with a synthesis of our results and a discussion of how the analysis might be applied and extended to other systems.

2 Models

We will examine different ways in which sensing occurs, and how the sensed signal may be propagated downstream. In this section we discuss the modules describing how the sensed signals are propagated. Our modules are intended to be compact representations of different characteristic signal transduction observed in different systems. For the most part we will be concerned with spatial signalling mechanisms. However purely temporal signalling mechanisms are also contained in two of the models (discussed later). In general, for the modules we consider, chemical signalling occurs through receptor-ligand binding where the receptors could be evenly distributed along the membrane, or localized in some sub-region. For simplicity, all these modules are formulated on a 1-D spatial domain with periodic boundary conditions, representing the boundary of a cell. The main insights are unchanged by a change of domain or boundary conditions. Schematic diagrams of the three modules which we discuss are shown in Fig. 1. Our qualitatively simplified models are intended to capture key aspects of how sensed signals are connected to downstream components which regulate F-actin. Thus the output may be regarded as a representative biochemical “frontness” component or component which biases pseudopod extension (eg. PIP_3 or Rac).

The first model involves a simple description of local regulation. Local regulation/biasing is the basis for description of migration in certain cell types (Arriemerlou & Meyer, 2005), and can be expected to be important in cells which are already polarized with an existing pseudopod/front, driving the migration. In this model, a signal S regulates the production of an active form of a response element R^* from an inactive form R . The equations governing the response are given by

$$\begin{aligned}\frac{\partial R^*}{\partial t} &= (k_f + kS)R - k_r R^* + k_d \frac{\partial^2 R^*}{\partial \theta^2} \\ \frac{\partial R}{\partial t} &= -(k_f + kS)R + k_r R^* + k_d \frac{\partial^2 R}{\partial \theta^2}\end{aligned}\quad (1)$$

In the above equations k_f and k_r denote the rate constants for the constitutive conversion between inactive and active forms. k is the rate constant involved in the signal mediated conversion of inactive to active forms. Finally k_d is the diffusion coefficient of the active and inactive forms (assumed equal for simplicity). The equations are written in dimensionless form, with $R + R^* = 1$ initially. By adding the above two equations we see that

$$\frac{\partial(R + R^*)}{\partial t} = k_d \frac{\partial^2(R^* + R)}{\partial \theta^2}\quad (2)$$

so that if $R + R^* = 1$ initially, this condition holds for all time and so we can write $R = 1 - R^*$. Note that the signal affects the forward reaction here. An exact analogue involves the signal causing the degradation

of R^* rather than the production. This results in

$$\frac{\partial R^*}{\partial t} = k_f(1 - R^*) - (k_r + k_S)R^* + k_d \frac{\partial^2 R^*}{\partial \theta^2} \quad (3)$$

where the above conservation condition is explicitly incorporated. Note that in the above equation, restricting the signal to be homogeneous (or as occurring through localized receptors) and setting $k_d = 0$, we have a model of local temporal sensing.

The second model which we consider includes adaptation as an important ingredient of sensing. This is motivated by the demonstrated presence of adaptive signal transduction to different pathways such as PI3K and PTEN in *Dictyostelium*. It should be noted that the temporal adaptive behaviour is combined with a non-trivial spatial gradient sensing response. The essential ingredient for this to occur is some regulatory pathway which is not purely local, and could for instance be a highly diffusible element (Levchenko & Iglesias, 2002; Iglesias & Devreotes, 2008). Other ways of giving rise to similar effects through cytosolic pools is discussed in (Skupsky *et al.*, 2005). For our purposes we will employ a compact model which gives rise to adaptive signalling and spatial responses. Since, we will be considering both attractive and repulsive responses, we choose a generalization of the local excitation global inhibition model developed previously (Krishnan, 2009). This involves a response element R^* to be regulated by an activator and inhibitor both of which may be diffusible. For simplicity, we will assume the response element to be non-diffusible. Again, similar to the above, a conservation condition holds good between the inactive and active forms of the response element. Incorporating this, the governing equations for this model are

$$\begin{aligned} \frac{\partial A}{\partial t} &= k_a S - k_{-a} A + k_{da} \frac{\partial^2 A}{\partial \theta^2} \\ \frac{\partial I}{\partial t} &= k_i S - k_{-i} I + k_{di} \frac{\partial^2 I}{\partial \theta^2} \\ \frac{\partial R^*}{\partial t} &= (k_f) A (1 - R^*) - k_r I R^* \end{aligned} \quad (4)$$

Here A and I denote the concentrations of activator and inhibitor respectively, while R^* denotes the concentration of the response element. Here k_a and k_{-a} denote the activation and deactivation rate constants for the activating enzyme, while k_i and k_{-i} denote the activation and deactivation rate constants of the inhibiting enzyme. The activating and deactivating rate constants of the response element are denoted by k_f and k_r respectively. The diffusion coefficients of the activator and inhibitor are denoted by k_{da} and k_{di} respectively. In this case when S is spatially homogeneous, the response R^* is independent of S , as it depends on the ratio of A and I , both of which are proportional to S . Note that here too, by setting

$k_{da} = k_{di} = 0$ and restricting the signal to be independent of space (or as occurring through localized reception) we have a case of purely temporal sensing with adaptation.

The third model embodies another characteristically different form of signal transduction which is observed in certain eukaryotic systems like leukocytes: spontaneous polarization. The model we employ for this purpose is based on the work of Narang (Narang, 2006) (which in turn is based conceptually on (Xu *et al.*, 2003)), and involves spatial sensing with the special feature of spontaneous polarization induced by spatially homogeneous signals. In this model, the addition of a spatially homogeneous stimulus leads to symmetry breaking via a Turing instability, leading to a well defined front and back. For our purposes, the main features of interest in this model are that it leads to spontaneous symmetry breaking and also that the frontness and backness signals are present in an explicit manner. The fact that the symmetry breaks via a Turing instability is of much less relevance.

This model involves 3 components u_1 (the cytosolic inhibitor, also modelled in 1-D for simplicity), the frontness component u_2 (representative of Rac/Cdc42) and the backness component u_3 (representative of Rho). This model is based on the mutual inhibition of frontness and backness components both directly and through the upregulation of the intermediate cytosolic component u_1 . The receptor signal upregulates both the frontness and backness components. The model equations are

$$\begin{aligned}\frac{\partial u_1}{\partial t} &= -u_1 + a_{12}u_2 + a_{13}u_3 + D_1 \frac{\partial^2 u_1}{\partial \theta^2} \\ \frac{\partial u_2}{\partial t} &= \rho_2 u_2 (S - a_{21}u_1 - a_{22}u_2 - a_{23}u_3) + D_2 \frac{\partial^2 u_2}{\partial \theta^2} \\ \frac{\partial u_3}{\partial t} &= \rho_3 u_3 (S - a_{31}u_1 - a_{32}u_2 - a_{33}u_3) + D_3 \frac{\partial^2 u_3}{\partial \theta^2}\end{aligned}\quad (5)$$

In the above equation, the regulation of cytosolic component u_1 by the frontness and backness components is described by the rate constants a_{12} and a_{13} respectively; the first term on the right hand side of the first equation describes the constitutive degradation of this cytosolic component, while D_1 describes its diffusion coefficient. In the second equation a_{21} and a_{23} are rate constants which depict the inhibitory effects of the cytosolic inhibitor and the backness component on the frontness component. The frontness component is regulated by the signal, but also involves constitutive degradation (associated with the term a_{22}). D_2 is the diffusion coefficient of the frontness component. The terms in the third equation are described in an exactly analogous fashion. ρ_2 and ρ_3 are scaling constants which arise when the equation is non-dimensionalized. Further details are given in (Narang, 2006). In the above equation, typically u_1 is highly diffusible. Note that the cytosolic pool u_1 is produced by both u_2 and u_3 and plays a role in inhibiting each of these components (see Fig. 1 for a schematic). A degradation of each component u_2, u_3

which is quadratic is assumed. The essential aspect of the interaction of u_2 and u_3 is similar to the Lotka-Volterra equation. The difference in diffusivities of the species plays a crucial role in the Turing mechanism. Analytical results demonstrating the presence of the instability, and dependence on parameters are presented in (Narang, 2006). While this model was originally formulated with Neumann boundary conditions, we will employ periodic boundary conditions. This introduces no qualitative difference.

The above models are analyzed both analytically and numerically: simulations are performed by discretizing space, and solving the resulting equations in MATLAB using the ODE solver ode15s. Sample parameter values are employed. In the case of both the adaptive and the Narang module, some comments must be made about the choice of parameters. In the adaptive module, a response whose gradient response parallels the upstream signal is achieved when $k_{da}/k_{-a} < k_{di}/k_{-i}$ and a repulsive response is observed in the opposite case. For specificity we choose parameter values which lie in the parameter region $k_{da}/k_{-a} < k_{di}/k_{-i}$. Our main conclusions, being qualitative in nature, do not depend on the particular choice of parameters. In the Narang module, we employ a basal set of parameters taken from (Narang, 2006). In this module, under basal conditions, the spatially homogeneous state is stable, but may be destabilized (leading to an inhomogeneous state) when the signal level crosses a particular threshold. The resulting patterned state is reminiscent of a polarized cell, and depending on the choice of parameters in the module, the resulting state (just above the instability threshold) may be either representative of an attractive response (i.e. frontness component highest near maximum of signal) or that of a repulsive response (i.e. frontness component highest near minimum of signal). Again for specificity we choose parameters so that the attractive response is obtained. We will comment in detail on the role of the parameters in this system in the context of specific results.

3 Analysis of models

In this section, we present various results related to the ways in which repulsive and attractive response may be obtained in the same cellular system. In order to do this, we examine two basic mechanisms postulated and partly studied in the literature: one which postulated that chemorepulsive signalling is related to chemoattractive signalling, via the opposite regulation of a key upstream component, which is referred to as a polarity switch. The second scenario is one where upstream competing pathways play a crucial role in the cell exhibiting chemorepulsion and chemoattraction. This is based on such pathways being observed in growth cones, and similar effects being observed in neutrophils. However in no case has

either scenario been examined carefully and systematically in light of the possible signal transduction which might occur. We will examine both scenarios, and pay particular attention to how these might work in different cells which have very different signal transduction characteristics. In order to do this, we examine how each of the scenarios described above would work to propagate signals downstream. This involves considering each of these scenarios upstream of each kind of signal propagation module. Analyzing each of these settings allows us to make robust conclusions regarding the roles of polarity switches and competing effects in spatial gradient sensing and chemotactic signalling.

Before we examine this, we first briefly analyze the different basic modules embodying local/simple feedforward regulation, adaptive signal transduction and spontaneous polarization.

3.1 Response of signal propagation modules

We first investigate the simple local model (see Fig. 2). The imposition of a linear gradient ($S = a + b \cos \theta$ in terms of the angular co-ordinate) results in a response which essentially mirrors the input. This is the simplest kind of module. There are a few points to be noted even in this simple module. Firstly, the response is essentially proportional to the input when this module is far from saturation. Secondly, the basal reaction rate constants determine the range over which the response can vary at steady state. In general, if the relevant basal reaction rate constant (forward for attractive biasing, backward for repulsive biasing) is small relative to the basal reaction rate constant of the opposite reaction, then practically the entire range of response element concentrations can be exploited. Thirdly while the regulating reaction is assumed to follow mass-action kinetics, alternate mechanisms such as Michaelis-menten kinetics can lead to a non-linear distortion of the input signal. Finally any threshold mechanism downstream can also lead to a sharpening or simple amplification of the response.

Very similar insights apply to a local module of repulsive sensing. Here again the presence of a basal backward reaction rate can restrict the range over which the response may vary in response to the signal. Here, the presence of a threshold mechanism downstream could lead to a sharp falling off of net output as a function of signal concentration. In both these cases the effect of increasing the diffusion coefficient of the response element is to reduce the amplitude of variation of the response. As this diffusion coefficient is made larger the gradient information is gradually lost.

We now turn to the adaptive module (Fig. 2(b)). Again as before, we investigate the case of a linear gradient. In the first case, we use parameters such that $k_{da}/k_{-a} < k_{di}/k_{-i}$. The simulations performed revealed an attractive biasing with a response maximum coincident with the input signal maximum. In

general increasing the gradient strength increases the amplitude of the response, while a homogeneous signal of any magnitude keeps the response fixed. Further by varying the parameters, we see that the smaller the difference $k_{di}/k_{-i} - k_{da}/k_{-a}$ for a fixed input signal, the weaker the steady state gradient response. The presence of a downstream amplifying element allows for an amplification of this signal in such a way that the adaptation property is preserved (assuming this amplifying mechanism is via a monostable threshold mechanism). For completeness we investigate the behaviour of the module in response to a signal when $k_{da}/k_{-a} > k_{di}/k_{-i}$. In this case the response of the module is to produce a repulsive biasing. The same conclusions regarding the positive biasing case above hold good here.

Finally we investigate the Narang module, when subject to a gradient (see Fig. 3). For the parameters employed, a clear polarized response representative of chemoattraction is obtained. When the gradient is relatively weak, both the frontness signal u_2 and the backness signal u_3 display localized responses. When the gradient is substantially strong we clearly see that the frontness signal u_2 and backness signal u_3 also develop localized profiles in response to the gradient, though the effect of the gradient leads to the frontness signal being sharply localized and the backness component displaying a weakly bi-modal response. The basis of the underlying mechanism of this module is spontaneous symmetry breaking, leading to polarized profiles (Narang, 2006). A similar simulation of this module with different parameter values and the same input signals, reveals polarized profiles for the frontness and backness components u_2 and u_3 respectively, which are representative of a chemorepulsive response. In this case, for strong gradients, the backness signal is more sharply localized. Further, this chemorepulsive response also involves a spontaneous symmetry breaking mechanism. Thus this module, for different choices of parameters can result in either a chemoattractive or a chemorepulsive mechanism with spontaneous symmetry breaking in homogeneous stimulation.

These three modules act as representative modules of signal propagation, embodying very different behaviour, but still producing/propagating attractive or repulsive biasing. It is worth pointing out, incidentally, that both the adaptive as well as the Narang models can exhibit both repulsive as well as attractive biasing simply by changing parameters. We will return to this point later in the paper.

3.2 A polarity switch

In general it is worth noting that chemoattraction and chemorepulsion typically involve different chemical signals, and perhaps different receptors too. Therefore it is possible that chemoattractive behaviour and chemorepulsive behaviour could occur through very different pathways, involving qualitatively completely

different mechanisms. In this subsection, we examine a scenario which has been postulated and discussed in the literature: the opposite regulation of a key upstream element by chemoattractant and chemorepellent is suggested to be the key to the opposite response in the system.

Here we will examine this possibility in more detail. In particular we will examine what such a simple polarity switch implies for the downstream response, and also what kind of signal transduction might be consistent with the possibility of a simple upstream polarity switch. In order to do this, we will examine how an upstream polarity switch would work along with essentially linear/simple feedforward signal transduction, adaptive signal transduction and signalling involving spontaneous polarization.

In order to address this issue we revisit the above models. We expand these models somewhat to include an additional intermediate reaction whose output S^* is the input to the module, rather than the receptor signal S itself (see Fig. 4). Thus S^* is the input to the module, and its dynamics are described by

$$dS^*/dt = (k_1S + k_{f1})(1 - S^*) - k_{r1}S^* \quad (6)$$

in the case of activation, and

$$dS^*/dt = (k_{f1})(1 - S^*) - (k_1S + k_{r1})S^* \quad (7)$$

in the case of inhibition (note that it is assumed that $S_0 + S^* = 1$ where S_0 is the inactive form of S^*). In the above, k_{f1}, k_{r1} are the basal activation and inactivation rate constants for the regulation of S^* . k_1 is the rate constant associated with the receptor mediated activation/deactivation of S^* . In other words, the receptor regulatory pathways act on the key upstream element S^* , and this upstream signal controls the regulatory dynamics of network. Thus S^* plays the role of a potential polarity switch.

We can immediately make a few conclusions from the above model. Firstly we note that if $k_{f1} \ll k_{r1}$ then the equilibrium when $S = 0$ in the above reaction is very much towards the left. A corresponding conclusion can be made when $k_{r1} \ll k_{f1}$. Now we investigate whether the reversal of regulation of this common element will effectively revert the nature of the downstream response. To do this we first note that if either of the rate constants k_{r1}, k_{f1} is very small relative to the other, then this results in a basal value of S^* either very small or close to saturation. This severely limits the dynamic range in one direction, and so in our simulations we will assume that both constants are of comparable magnitude, leading to an equilibrium neither too much to the left nor to the right.

We first investigate the local module. Fig.5 compares the case where the activation in the common element is switched to inhibition. We see a clear switch in the downstream response from attraction to

repulsion with local sensing. Another related point should be mentioned. If this local sensing module has a downstream threshold module (monostable, local) which leads to non-linear amplification, then the reverse regulation of S^* , even if it leads to a repulsive biasing cannot employ this threshold effect, as it generally pushes the response everywhere further away from the threshold.

The conclusion from this study reveals that a simple polarity switch can lead to a reversal in downstream gradient response. However this comes at a price: the opposite regulation of a common upstream element means that any downstream (monostable) threshold can be employed only by one of the two opposite regulatory effects of the polarity switch. Nevertheless it is possible that such a scenario may work in cells which are already polarized and migrating through essentially local gradient response to pre-existing pseudopods/growing fronts (Krishnan & Iglesias, 2007; Arriemerlou & Meyer, 2005).

We now investigate the effect of this reverse regulation of S^* if the downstream module is an adaptive module. Fig.5 shows the net result. The effect of the reversed regulation of S^* results in the biasing being altered from attractive to repulsive behaviour. This is also seen by simple analytical results. We note here that the response to the adaptive module to a homogeneous signal is independent of the value of the input signal, or indeed of the basal value of S^* . We further note that both the spatial as well as temporal behaviour of the response are reversed. Thus in homogeneous stimulation, the response is switched from a transient jump to a transient depression before recovery to basal levels (an exploration of a “polarity switch” in the context of a purely temporal adaptive signalling model is made in the Appendix).

In summary, if the downstream signal transduction is adaptive (with spatial sensing), then an upstream polarity switch results in the downstream response being reversed, in a manner consistent with adaptation in homogeneous signalling. An implication of such downstream adaptive signalling is that it allows an upstream polarity switch to provide the appropriate downstream biasing in a way which keeps the mean value fixed. Thus, unlike in the simple local regulation, a polarity switch no longer implies that the downstream signal is regulated in opposite directions relative to basal values. This also means that any downstream monostable threshold may be accessed for both the opposite regulation of the polarity switch.

While we have used a specific module of adaptive signalling with spatial sensing, we note that this result is more general, and applies to any similar adaptive signalling which involves a global regulatory element, which serves to compensate for changes in the mean value of the upstream signal. Thus downstream adaptive signalling is in many ways advantageous for the functioning of a polarity switch.

We now examine how such an upstream polarity switch would work in conjunction with signalling involving spontaneous polarization (Fig. 6). Here too we find that changing the regulation of S^* from

activation to inhibition alters the nature of the downstream response from attractive to repulsive. However a closer look at the response reveals a rather different response when this signal is reversed, and in general the localization of u_2 and u_3 is much less sharp.

Some basic analysis of this module reveals the main difference. While the attractive response involves spontaneous polarization (via the Turing mechanism in this case) as a crucial ingredient in the gradient sensing response, switching the regulation of S^* results in a situation where no spontaneous polarization is observed. Indeed, in this case, the parameters in the Narang module are regulated so as to move further away from the instability/spontaneous polarization threshold, rather than cross it. Thus the repulsive response obtained is qualitatively fundamentally different from the attractive response. Thus if the response to a chemorepellent involved a simple inhibition (as opposed to activation) of a common upstream component, this would necessarily involve a completely different kind of sensing response.

To complete one aspect of the analysis of the Narang module above, it is worth asking whether an alternate regulation of the upstream component S^* would result in a chemorepulsive response which would also involve spontaneous polarization as a key ingredient. We show using one scenario that this is indeed possible. Indeed if the regulation of S^* is locally inhibited by the signal, and simultaneously also activated by a signal which is related to the spatial average of the signal, this is indeed possible. This latter activation could be through some pathway involving highly diffusible components. To illustrate this point we consider alternate regulation of S^* as

$$\begin{aligned} dS^*/dt &= (k_{f1} + k_{11}A)(1 - S^*) - (k_1S + k_{r1})S^* \\ dA/dt &= k_{fa}S^2 - k_{ba}A + k_{da}\frac{\partial^2 A}{\partial \theta^2} \end{aligned} \quad (8)$$

where A refers to the additional pathway which is highly diffusible.

Now the results in Fig.6(c) show that a repulsive response is indeed obtained, with a sharper localization of frontness and backness components. Some further analysis reveals that this indeed involves spontaneous symmetry breaking as an ingredient in the gradient response. A look at the above equation reveals the main issue: the spontaneous symmetry breaking involves pushing the input to the Narang module past a particular threshold. In this above modification, for a given input signal S the gradient information causes the spatial biasing to be opposite to that of attraction. In addition, the regulation of the activation while providing no gradient information, allows for S^* to be increased (even counteracting the decrease due to the local inhibition) so that the threshold may be crossed for strong enough input signals. The above combination of factors allows for a repulsive response which also exhibits spontaneous

polarization in homogeneous signalling and is a basic ingredient in the gradient response.

In essence the above analysis suggests the following conclusions. A simple upstream polarity switch in a system which exhibits spontaneous polarization as a key element in the gradient response will indicate a pronounced difference in the attractive and repulsive responses. Firstly, no spontaneous polarization may be expected in homogeneous chemorepellent stimulation, and the gradient response is likely to indicate a much weaker polarization. Further it also suggests that a chemorepellent can even depolarize a cell. Thus for instance a homogeneous stimulation of chemoattractant can lead to spontaneous polarization; however if the cell is subsequently subjected to a homogeneous dose of chemorepellent of sufficient strength, then this has the effect of directly working against the chemoattractant, and regulating the upstream element in the opposite direction, and pushing it below the stability threshold of the downstream module. Thus, in such a case, a homogeneous stimulation of chemorepellent can depolarize a polarized cell.

While we have used the Narang module for illustrative purposes, we note that this conclusion holds for any similar module which exhibits spontaneous polarization due to some upstream regulation pushing it past the stability threshold. If the signalling to the spontaneous polarization module occurs primarily through one pathway (for eg. PIP_3) then the opposite regulation of this pathway by chemorepellent will necessarily push it away from the stability boundary. We will discuss the implications of this point later.

Taken together, our analysis reveals that while a simple upstream polarity switch can result in the reversal of the biasing, the downstream signal transduction characteristics can play a crucial role in how such a switch might function to provide an opposite gradient sensing response.

3.3 Multiple and competing effects in gradient sensing

In the previous subsection, we examined how a so-called polarity switch could result in the reversal of the sensing but with very different characteristics. In this subsection, we examine a different scenario, also motivated directly by experiments, namely the possible presence of multiple and competing effects in gradient sensing. This is related to examining whether signal transduction to the same chemical can lead to attractive and repulsive responses under different conditions. We will consider the case of a gradient response being transduced by multiple pathways, in competition with one another.

Multiple competing effects are observed in very different cell signalling settings and related effects have been examined in the literature under the name hormesis (Calabrese, 2005; Calabrese, 2001). In the chemotactic context, the consideration of competing effects is very natural, since these have been observed in different systems, including growth cones and neutrophils (Song *et al.*, 1998; Vianello *et al.*, 2005). The

exact details of how these competing effects are integrated in these systems is still being elucidated.

We aim to examine what the consequences of having competing pathways in gradient sensing are, and what kind of signal propagation possibilities can result. In order to do this we examine a model of competing pathways which is stripped down to its essential features. Thus we examine the parallel feedforward regulation of two pathways corresponding to species A and B whose respective total amounts are A_{tot}, B_{tot} . A and B represent the multiple elements in signal transduction. Both active A and B regulate a response element R^* from its inactive form. For simplicity, the response element is assumed non-diffusible. This leads to the equations:

$$dR^*/dt = k_f(A + B)(R_{tot} - R^*) - k_r R^* \quad (9)$$

This equation describes the conversion to active form of the response element by the pathways A and B: the associated forward rate constant is k_f and a constitutive backward reaction with rate constant k_r is assumed. In the above description the total amount of active and inactive forms of the response element is a constant (and equal to R_{tot}) and this has been explicitly incorporated in the dynamical description. Note that in general different rates may be assumed for the regulation of the forward reaction associated with A and B, but this effect can be captured essentially in the total amounts of A and B. The regulation of A by the signal leads to

$$\frac{dA}{dt} = (A_{tot} - A)(k_{fa} + k_a S) - (k_{ba})A + k_{da} \frac{\partial^2 A}{\partial \theta^2} \quad (10)$$

for the case where A is positively regulated by the signal. Note that A refers to the active form of the enzyme A. In this equation (and similarly below), k_{fa} and k_{ba} denote constitutive reaction rate constants for the activation and deactivation of the active form of A. k_a denotes the rate constant associated with the upstream signal regulation of A (in the above, this regulation occurs for the positive reaction). k_{da} denotes the diffusion coefficient of the active form of A (assumed to be the same as that of the inactive form). Since the concentrations of the active and inactive forms satisfy a conservation condition (owing to the fact that there is only interconversion between active and inactive forms, and that their diffusion coefficients are equal), this is explicitly incorporated above. The equations for the other cases is presented below, in an exactly analogous notation. In the case of negative regulation of A by the signal, we have

$$\frac{dA}{dt} = (A_{tot} - A)(k_{fa}) - (k_{ba} + k_a S)A + k_{da} \frac{\partial^2 A}{\partial \theta^2} \quad (11)$$

In an exactly analogous way, the regulation of B by the signal leads to two possibilities:

$$\frac{dB}{dt} = (B_{tot} - B)(k_{fb} + k_b S) - k_{bb}B + k_{db} \frac{\partial^2 B}{\partial \theta^2} \quad (12)$$

in the case of activation and

$$\frac{dB}{dt} = (B_{tot} - B)(k_{fb}) - (k_{bb} + k_b S)B + k_{db} \frac{\partial^2 B}{\partial \theta^2} \quad (13)$$

in the case of inhibition.

This leads to four possible effects of the coregulation of the pathways: (i) Forward regulation of both A and B (ii) Backward regulation of both A and B (iii) Forward regulation of A and backward regulation of B and (iv) Backward regulation of A and forward regulation of B. We examine these in turn. A schematic diagram showing the positive regulation of A and negative regulation of B is shown in Fig. 7.

Sample simulations of each of these cases is shown in Fig. 8. In Fig. 8(a) we see the simple additive effects leading to positive biasing in a signal. In Fig. 8(a) we also see the parallel effects of two pathways leading to negative biasing. In Fig. 8(b) in contrast there is a competition between A and B, and the net effect is a positive biasing of the signal suggesting a dominance of A for the conditions considered. Also shown in this figure is a case of negative regulation of A and positive regulation of B resulting in the net negative biasing, suggesting the dominance of A under these conditions.

While the results of cases (i) and (ii) are intuitively obvious, the case of (iii) and (iv) are of interest. We will examine the case of (iii), which directly reflects the case of competing pathways in feedforward regulation. For simplicity, we start by considering A and B to be non-diffusible and the case where the basal forward rate constant k_{fa} and the basal backward rate constant of B are zero. Further simulations exploring the competition between A and B are presented in Fig. 9. We perform some analysis below. Now the steady state response of the response R^* is dependent on the spatial profile of $A + B$. It is a simple matter to see that

$$A + B = \frac{A_{tot}}{(1 + (k_{ba}/k_a S))} + \frac{B_{tot}}{(1 + k_b S/k_{fb})} \quad (14)$$

The competing effects of the signal in each of the terms in the right hand side is clearly seen. In order to examine the nature of the response and its dependence on the signal, we examine when $(d/dS)(A + B)$ is greater than zero. We set $k_a/k_{ba} = K_1, k_b/k_{fb} = K_2$. Thus the variation of $A + B$ and hence the response with signal at steady state indicates positive biasing if

$$\frac{(1 + K_1 S)^2}{(1 + K_2 S)^2} < \frac{K_1 A_{tot}}{K_2 B_{tot}} \quad (15)$$

This equation reveals that depending on parameter values both positive and negative biasing may be obtained. In particular the left hand side is a monotonic function of S varying between 1 and $(K_1/K_2)^2$, while the right hand side depends on both the ratio K_1/K_2 as well as A_{tot}/B_{tot} .

We can immediately infer a few important points from the above. If $K_1 = K_2$, then positive biasing is always obtained if $A_{tot} > B_{tot}$ and negative biasing is always obtained if the opposite inequality holds. This is intuitively obvious. However when K_1 and K_2 are different for a fixed A_{tot}/B_{tot} neither too large not too small, the inequality above may be reversed at a fixed S . In this case, we have a switch from attractive to repulsive biasing as the signal strength crosses a particular level. The core of this effect is the competing effects of the pathways as well as saturation. This effect is explored in Fig.10 and Fig. 11.

We now examine the effect of the presence of basal forward/backward reactions (i.e. $k_a, k_b > 0$). In this case the steady state for A+B is obtained as

$$A + B = A_{tot}/(1 + (k_{ba}/(k_{fa} + k_a S))) + B_{tot}/(1 + (k_{bb} + k_b S)/k_{fb}) \quad (16)$$

Here again, exactly as above one can examine when the response shows a positive biasing towards the signal. In this case, the condition above is replaced by

$$\frac{(1 + [(k_{fa} + k_a S)/k_{ba}])^2}{(1 + [(k_{bb} + k_b S)/k_{fb}])^2} < \frac{A_{tot}k_a/k_{ba}}{B_{tot}k_b/k_{fb}} \quad (17)$$

Again this equation can be analyzed explicitly to see if this inequality is reversed for a specific value of S . This reveals a critical value of signal mean value at which the gradient response may be reversed. If we define Q as

$$Q^2 = (A_{tot}k_a/k_{ba})/(B_{tot}k_b/k_{fb}) \quad (18)$$

then the critical mean value at which the gradient response is reversed is

$$S_{crit} = \frac{(1 + k_{fa}/k_{ba}) - (1 + k_{bb}/k_{fb})Q}{k_b/k_{fb}Q - k_a/k_{ba}} \quad (19)$$

Fig. 10 shows different cases of the variation of the response when the pathways are subject to a homogeneous stimulus. In particular this reveals that the response can be either a monotonically increasing function of the signal strength, a monotonically decreasing function, an initially increasing function, which reaches a maximum and starts decreasing or an initially decreasing function which reaches a minimum and starts increasing. Since the pathways are non-diffusible these plots also provide information about the nature of the response in a gradient: this response can be either always a positive biasing, always a negative biasing, or a response which can switch from a positive to a negative biasing at particular signal strengths.

So far we have examined the response of the two competing pathways, and examined when the net response is biased towards and away from the signal. In the above analysis the species were assumed to be

non-diffusible. Now we re-examine this analysis for the case where both A and B diffuse. This is important while considering spatially varying signals. Our goal is to gain some basic analytical insight into how the results above are affected by diffusion. While exact analytical results are difficult, we can gain useful insights by examining the case of weak gradients. Thus we will examine the case where the input signal is $S = S_0 + \epsilon S_1 \cos\theta$ (a linear gradient imposed on a circular cell) where $\epsilon \ll 1$ and S_0, S_1 are constants. Here S_0 represents the mean value of the signal and ϵS_1 represents the gradient strength. ϵ is a scaling factor. The case $\epsilon \ll 1$ can be analyzed analytically. In order to do this, we go back to the governing equations for A and B, and expand them as a regular perturbation series in ϵ :

$$\begin{aligned} A &= A_0 + \epsilon A_1 + \epsilon^2 A_2 + \dots \\ B &= B_0 + \epsilon B_1 + \epsilon^2 B_2 + \dots \end{aligned} \quad (20)$$

Now, the various terms can be obtained by plugging these expressions into the governing equations with the above choice of signal, and equating terms with like powers of ϵ . This leads to

$$\begin{aligned} A_0 &= A_{tot} \frac{k_{fa} + k_a S_0}{k_{fa} + k_a S_0 + k_{ba}} \\ B_0 &= B_{tot} \frac{k_{fb}}{k_b S_0 + k_{bb} + k_{fb}} \end{aligned} \quad (21)$$

Naturally these leading order terms do not capture the gradient information. That is contained in the first order terms. These are obtained from the equations

$$\begin{aligned} k_a S_1 \cos\theta (A_{tot} - A_0) + (k_{fa} + k_a S_0)(-A_1) - k_{ba} A_1 + k_{da} \frac{\partial^2 A_1}{\partial \theta^2} &= 0 \\ k_{fb}(-B_1) - (k_{bb} + k_b S_0)(B_1) - k_b S_1 \cos\theta B_0 + k_{db} \frac{\partial^2 B_1}{\partial \theta^2} &= 0 \end{aligned} \quad (22)$$

These are linear inhomogeneous equations for A_1, B_1 and their solution is obtained by using a Fourier series, which in this case amounts to setting $A_1 = A_{11} \cos\theta, B_1 = B_{11} \cos\theta$ and solving for A_{11}, B_{11} . This leads to their solution

$$\begin{aligned} A_{11} &= \frac{k_a S_1 (A_{tot} - A_0)}{k_{fa} + k_a S_0 + k_{ba} + k_{da}} \\ B_{11} &= \frac{-k_b S_1 B_0}{k_{fb} + k_{bb} + k_b S_0 + k_{db}} \end{aligned}$$

Thus to leading order

$$\begin{aligned} A &= A_{tot} \frac{k_{fa} + k_a S_0}{k_{fa} + k_a S_0 + k_{ba}} + \epsilon A_{tot} \cos\theta \frac{k_a k_{ba} S_1}{(k_{fa} + k_a S_0 + k_{ba} + k_{da})(k_{fa} + k_a S_0 + k_{ba})} \\ B &= B_{tot} \frac{k_{fb}}{k_b S_0 + k_{bb} + k_{fb}} - \epsilon B_{tot} \cos\theta \frac{k_b S_1 k_{fb}}{(k_b S_0 + k_{bb})(k_{fb} + k_{bb} + k_b S_0 + k_{db})} \end{aligned} \quad (23)$$

The net behaviour of the internal gradient response depends on the sum of the last terms of each equation (which are of opposite sign). If this sum is positive, then this indicates a positive biasing, otherwise the response represents a negative biasing. This net behaviour can be examined in different limiting cases. Note that the gradient behaviour depends on the diffusion coefficients as well as the mean value of the signal.

The combined response of the pathways A and B in the case where both are non-diffusible, and where either or both may be diffusible raises some important points which we discuss now. Recall that from our previous discussion (when $A_{tot} > B_{tot}$) that when the signal S is increased, the combined response evolves in either a monotonic way across the entire range of signals or in a non-monotonic way. Thus the curve of the response vs signal provides crucial information. It also indicates the absolute range of response, which provides other useful information.

Now when the pathways A and B are diffusible, we may expect a perturbation of the results obtained for non-diffusible pathways. This is indeed the case when the diffusion coefficients of A and B are small. Examining the above expressions in some special cases proves illuminating. In particular, suppose A is highly diffusible $k_{da} \gg 1$ and B is hardly diffusible. Then it is easily seen that the gradient response is always repulsive—this is because it is pathway B which provides the gradient information. Now the behaviour of the total response in a homogeneous signal may either be monotonic (always increasing or always decreasing) or may even change sign as seen in the analysis above. However because of the effect of diffusion, the gradient response is always negative. When A is non-diffusible and B is highly diffusible, we have the opposite case and here the gradient response is always attractive whatever the variation of the response in homogeneous signals is.

The above point also indicates how, by virtue of diffusion, it is possible to substantially distort the results of the non-diffusive case and even essentially decouple the mean response and the gradient response. This point is very important when one notes that many threshold phenomena rely on the local concentration of a downstream element crossing a particular value. This analysis shows that it is possible to get different kinds of variation to homogeneous signals, both monotonic and non-monotonic, and have in some cases, a completely decoupled gradient response with two pathways. This also shows that in general one could have quite complex and unintuitive behaviour arising from this precise feature.

Downstream signal propagation. We now consider the effects of competing pathways on different kinds of downstream signal propagation. Firstly if there is simple local downstream regulation, then the main insights are contained in the analysis above. In particular, the possibility of the reversal of a gradient response when the mean value of the signal is increased, exists. Now if there is downstream adaptive

signalling then this can be analyzed by “connecting” the upstream competing pathways module with an adaptive module. In this case the downstream adaptive module simply acts to reset the level of the response without distorting the essential features of the gradient response. Thus a positive biasing is simply converted into another positive biasing response, with its level adjusted by the adaptive response, and similarly for a negative biasing. Other main features such as a reversal in gradient response also continue to hold good. Note that in this case both competing pathways are upstream of the adaptive signalling module.

We now examine the effect of a downstream module of spontaneous polarization in signal transduction with competing pathways. To illustrate this we “connect” the competing pathways module to the Narang module. Here the situation is a little more subtle. For the parameters in the Narang module chosen, the gradient response reflects that of the upstream signal. However the essential nature of the response also depends on whether the spontaneous polarization threshold has been crossed. It is simplest to examine the response in a weak gradient.

With this in mind, we first examine the nature of the response in homogeneous stimulation. Now if the input signal is homogeneous, we saw above that the response from the competing pathways module could either monotonically increase, increase and then decrease and reach an asymptotic value, monotonically decrease, or decrease and then increase. If the response monotonically decreases, then the threshold is never crossed and no (spontaneous) polarization occurs. Now if the response monotonically increases then it is possible that at some finite signal strength, the threshold is crossed, leading to spontaneous polarization. Any signal whose strength is above this value will always elicit such a response. If the response of the competing pathways module increases, reaches a maximum and then decreases, then if the maximum is above the spontaneous polarization threshold, spontaneous polarization will occur for a homogeneous signal of sufficiently high strength. Here however there exists the possibility that for signal of even higher strength, the response of the competing pathways module may decrease sufficiently so as to fall below the threshold of the spontaneous polarization threshold. This then means in response to a second stimulus of sufficiently high strength, depolarization may occur leaving an unpolarized cell.

We can build on these insights and consider the response of the combined modules to a weak gradient. To start with we consider the competing pathways to be essentially non-diffusible. The response of the system then depends on the mean value of the signal. If the mean value of the signal is low then an attractive gradient response will be observed. However if the mean value is high enough, the system can exhibit a strongly polarized attractive response, by virtue of the fact that the spontaneous polarization threshold is crossed. However depending on the characteristics of the competing pathways module as

discussed above, different kinds of responses may be observed. Essentially the mean value of the input signal (along with the characteristics of the competing pathways module) will determine if the threshold for spontaneous polarization is crossed or not. Further the slope of the curve of the response of the competing pathways module as a function of signal strength (in homogeneous signals) plotted above will determine whether the input to the spontaneous polarization module is co-aligned or counter-aligned to the gradient signal to the cell. Thus a monotonic curve above would correspond here to an attractive gradient response, with the additional feature of strong polarization if the threshold for spontaneous polarization is crossed. If the curve is non-monotonic then if the ascending part of the curve crosses the level corresponding to spontaneous polarization the response for signal levels where the curve is above the spontaneous polarization threshold will correspond to a highly polarized cell: the polarization of the cell will be co-aligned with the external gradient (i.e. frontness signal highest where signal is maximum) if the slope of the curve is positive, and the polarization and gradient response will be counter-aligned if the slope of the curve is negative. Thus to summarize, the response of the network to a weak gradient will depend on two factors which can be obtained from the curve: whether the response value for the competing pathways model is above or below the spontaneous polarization threshold, and also on the slope of the curve (see Fig. 12 for illustrative results). Such analysis also follows for pathways which may be diffusible. The only point to note here is that the diffusivity of the pathways can affect the response, and this effect can be seen from the analytical results and the discussion above.

In summary if one considers a sequence of experiments where the cells are subject to signals where the mean value of a signal is changed keeping the gradient strength fixed, one could have different kinds of transitions. Thus overall rather subtle combinations of gradient response (with or without spontaneous polarization) may be obtained by competing effects in gradient sensing.

3.3.1 Modulating the effects of competing pathways

The above subsection examined the signal transduction arising from two competing pathways and the particular focus here was to characterize the response to a range of signal values. In this subsection, we build on this to address how these pathways may be modulated by factors other than the external signal itself. It should be noted that the two pathways involve different elements which may be modulated by the cells depending on their internal states (eg. stage in the developmental cycle) or other factors in the growth or external medium (Nishiyama, 2003; Song *et al.*, 1998). It is also possible that other signal transduction in the cell may involve sequestration/uptake of some elements in the pathways. We will assume that in all

these cases, that the distortion to the pathways occurs at a fast time scale, and that the system has reached a steady state, where the system may now be characterized by new parameters A_{tot}, B_{tot} . Thus in effect these extra modulations change parameters of the network.

We first examine the case of the competing pathways module alone. We note that for any fixed set of parameters, the dose response curve of the network (for homogeneous stimuli) is either monotonically increasing, monotonically decreasing, initially increasing reaching a peak and then decreasing or initially decreasing reaching a minimum and then increasing. We note from this that a change/modulation in the strength of some pathway can completely change the dose response curve from any one of these four types to potentially any other type.

The implication of this point is the following. Changes in internal or external conditions can completely alter the nature of the dose response to the same signal. Thus even the gradient response of the cell can be completely changed by such modulation (see Fig. 11(c)). Therefore it is possible—because of an altered internal state of the cell or other conditions in the medium— to completely alter the nature of the gradient response to the same signal, as well as how that depends on the signal level. For example a cell which could exhibit only an attractive response can exhibit an attractive response which is transformed into a repulsive response at higher signal values. Other transitions can likewise be possible. These effects are primarily because the balance of the competing effects can be altered.

Similar effects can be examined when one analyzes competing effects with downstream polarization. Here again, modulating the strengths of different pathways can completely alter whether attractive, repulsive or both responses may be obtained, just as discussed above. In addition the altering of parameters in the competing pathways module can also alter the range of the dose response curve (even if the shape is not changed). This could have profound implications for whether the polarization threshold is crossed or not, for a fixed strength of the external signal. Thus the modulation of the strengths of the competing pathways could alter the gradient response from a highly polarized to a weakly polarized response, completely change the kind of gradient response (attractive or repulsive) or a combination of both.

The above indicates that while performing experiments and analysis on cellular response, one must be very careful in considering cells with essentially the same internal state as well as growth/external conditions. Differences in such effects can play important roles when non-trivial competing effects exist in gradient sensing, and even more so if symmetry breaking polarization mechanisms are additionally at play.

3.4 Spontaneous polarization and the attractive and repulsive response

In previous subsections, we analyzed the effect of signalling scenarios which could allow a cell to exhibit attractive and repulsive biasing and how this would work when the cell had the capacity to spontaneously polarize in homogeneous stimuli. Such behaviour is observed in different cells such as T-cells and neutrophils. In this subsection, we aim to flesh out in a little more detail, various relevant aspects. We start by noting that the polarization in these cells involves the RhoGTPase signalling circuit including Rac, Rho and (where applicable) Cdc42. Spontaneous polarization is expected to result primarily from the interaction of these components. From our perspective we expect that the difference in attractive and repulsive sensing is already manifest upstream of this circuit. In our analysis we have used a simplified model of spontaneous polarization, the Narang module, to analyze various issues.

Firstly, as examined above, if chemoattractant and chemorepellent regulate opposite reactions upstream then while stimulation of one of them can employ the spontaneous polarization (instability) mechanism, the other typically will not. While our analysis was based on the Narang module, it is basically valid for a polarization module with a single instability or non-linear dynamic threshold (eg. (Jilkine *et al.*, 2007; Dawes & Edelstein-Keshet, 2007)) with the regulation of a common single upstream component by attractant and repellent.

In the context of the Narang module, we note that the upstream signal regulates both frontness and backness elements, and hence there are two pathways controlled by the upstream signal. A natural next question to ask is whether a chemorepellent can regulate the same two pathways in different strengths to give rise to spontaneous polarization with chemorepulsion, given that spontaneous polarization is obtained in the chemoattractant regulation. In the Narang module, the parameters which are involved in the signal regulation of the polarity circuit are contained in the equations for u_2 and u_3 . Changing the strength of the receptor regulation of the frontness component u_2 has the effect of proportionally changing the parameters $\rho_2, a_{21}, a_{22}, a_{23}$ while changing the receptor regulation of the backness component u_3 has the effect of proportionally changing $\rho_3, a_{31}, a_{32}, a_{33}$: this is related to how the original equations were non-dimensionalized (Narang, 2006). Now the condition of a Turing instability occurring giving rise to a polarized profile depends on the following condition: $0 < \frac{a_{22}}{a_{12}}(b_{32} - 1) < a_{21} - a_{31} < \frac{a_{33}}{a_{13}}(1 - b_{23})$ for the case where the polarity circuit is wired to give an attractive response relative to its input signal: $b_{23}b_{32} > 1$, with $b_{23} < 1$ and $b_{32} > 1$. Likewise when the polarity circuit has parameters which give a repulsive response: $b_{23}b_{32} > 1$ along with $b_{23} > 1$ and $b_{32} < 1$, the instability occurs if

$\frac{a_{22}}{a_{12}}(b_{32} - 1) < a_{21} - a_{31} < \frac{a_{33}}{a_{13}}(1 - b_{23}) < 0$. In these equations $b_{23} = a_{23}/a_{22}$ and $b_{32} = a_{32}/a_{33}$. The point to note is that a change of the receptor regulatory pathways will affect the different parameters proportionally, as mentioned above, and hence leave the parameters b_{23}, b_{32} fixed. Noting the conditions above we see that the intrinsic proclivity of the circuit to provide an attractive or repulsive response relative to its upstream signal is in fact not altered (since b_{23}, b_{32} are fixed). The alteration of parameters a_{31}, a_{21} may cause the above inequality to be violated, and hence the relevant instability giving the polarized state to be suppressed. In summary an alteration of the receptor regulatory pathways is not sufficient to yield an instability mechanism with the opposite gradient response.

It is of course possible that in other polarity circuits, the signal can regulate the polarity circuit in multiple locations, and a chemorepellent may regulate the polarity circuit in different locations, or with different differential strengths, to result in spontaneous polarization. However such a setting has to, of necessity, be more complex than a simple upstream switch.

In the above, we were examining the case of cells subject to either different chemicals or different mean values of the same chemical. On the other hand if the internal state of the polarity circuit itself is modulated, either by intrinsic factors, or other external elements in the medium then it is possible to change the behaviour of the polarity circuit completely. Thus such a change can allow for the Narang module to fundamentally change its response so that its response represents repulsion to the same gradient signal.

The analysis above suggests that if chemoattraction and chemorepulsion to different chemicals involves a single upstream polarity switch, then homogeneous doses of each cannot both elicit spontaneous polarization which further has implications for the gradient response. This immediately brings up the question: suppose both chemoattractant and chemorepellent elicit spontaneous polarization in a particular cellular system, what can we infer about the signalling? One possibility is that the chemoattractant and chemorepellent act to modulate the polarity circuit in more than one location, and possibly different locations. This would suggest that the polarity circuit regulation may be quite different (in a very non-trivial way) in the two cases and further do so in a way to give rise to opposite biasing and spontaneous polarization. A second possibility is that opposite biasing is obtained upstream of the polarity circuit, but the mean value of this upstream signal is compensated so that the oppositely biased input to the polarization module also crossed the instability threshold.

Another possibility is that the polarity circuit regulation by the attractant is distinct or only partially overlapping with that regulated by the repellent. Noting that different cells have multiple isoforms of Rac and other RhoGTPases, we see that it is quite plausible that attractants and repellents might regulate

different forms of the same species. Thus different forms may be involved in different, or only partially overlapping polarity circuit interactions. In the context of the module we have analyzed, it is possible that two different isoforms of Rac (for eg.) could result in two different polarity circuits, one which is intrinsically “tuned” to give an attractive response and the other to give a repulsive response. The chemoattractant would regulate one polarity circuit and the chemorepellent would regulate the other polarity circuit. This is a possible way for obtaining spontaneous polarization in homogeneous stimulation of both chemoattractant and chemorepellent.

3.5 Polarity switch and competing pathways

In previous subsections we examined both the polarity switch and competing pathways, and the resulting signal processing in each case. Here, we briefly examine another issue which connects these two aspects. Our discussion of a polarity switch was based on the opposite regulation of an upstream component by chemoattractant and chemorepellent. For completeness, we discuss how this could be realized if competing pathways exist. Thus we examine the following situation: suppose a chemoattractant regulates downstream signalling through two competing pathways, could a chemorepellent also regulate the two competing pathways in the same manner but balanced differently to give rise to chemorepulsion?

Our analysis of the competing pathways immediately indicates that this is possible. Suppose A and B are the competing pathways (assumed non-diffusible for simplicity) then having a chemorepellent regulate these pathways amounts to changing the regulatory constants k_a, k_b . From our analysis above we see that a chemorepellent can regulate these competing pathways in a way in which the regulation of B is stronger than that of A, and this can result in a scenario corresponding to chemorepulsion. Furthermore, an analysis exactly along the previous lines suggests that since the response ends up below basal levels, any downstream spontaneous polarization mechanism will not be triggered in this case too.

An interesting point to note is that in this case both the $S = 0$ and the large S limits asymptote to the same values for chemoattractant and chemorepellent (as is revealed by a simple analysis). Thus suppose the dose-response curve for a chemoattractant is monotonically increasing, then in the chemorepellent case, the curve would initially decrease (indicative of chemorepulsion) but have to eventually turn around and increase. Thus one could predict that a chemorepellent regulation of the same pathways would result in a transition from repulsion to attraction as the signal mean value is increased. This is because the faster kinetic regulation of pathway B (which makes it dominate for relatively small levels of signal) is compensated by the greater “capacity” (or relatively slower saturation) of pathway A at higher signal

values. In general if in chemoattraction, the dose response curve is such that the large signal response is higher than the zero signal response, then the chemorepellent regulation of such competing pathways will involve a switch from repulsion to attraction as signal levels are increased.

4 Conclusions and Discussion

Attractive and repulsive migration in response to different kinds of cues is widespread in both prokaryotic and eukaryotic systems. Chemoattraction and chemorepulsion are specific examples of this. Interestingly many cells exhibit both chemoattraction and chemorepulsion—in particular eukaryotic cells such as *Dictyostelium*, neutrophils, T-cells and neural growth cones all possess this feature. It is likely that these opposite migratory responses have evolved to allow the cellular systems to accomplish specific responses. While it may be anticipated that both these phenomena will be the focus of many detailed modelling studies, there are other questions which arise at the outset. What are the design principles and features involved in the signalling networks of such cells which allow them to exhibit both chemoattractive and chemorepulsive responses? Are attractive and repulsive responses related by a simple upstream switch? How can an attractive response to a particular chemical be converted to a repulsive response? In this paper we develop a framework to analyze these questions (all related to the qualitative nature of signal transduction) from a more general non-system specific perspective. We believe that this provides a platform and systems-skeleton for guiding the examination of these issues in different specific systems, using detailed modelling and focussed experiments.

We examined two design principles which may allow cells to exhibit both chemoattraction and chemorepulsion, both directly motivated by the biological literature. One is that chemoattraction and chemorepulsion are related by simply the opposite regulation of an upstream component by attractant and repellent respectively. The opposite regulation of active enzyme PLC in *Dictyostelium* by chemoattractant cAMP and chemorepellent 8-CPT cAMP has been found experimentally (Keizer-Gunnink *et al.*, 2007). The other principle is that cellular signalling may have inbuilt competing effects upstream which allow it to exhibit both kinds of behaviour. Competing pathways (mediated by cAMP and cGMP) have been observed in neural growth cones, and are believed to be present in neutrophils and T-cells too (Song *et al.*, 1998; Vianello *et al.*, 2005). Competing effects could occur at the level of the receptor as well.

Eukaryotic cells typically sense their environment using a spatial sensing mechanism, and this leads the cell to polarize (form a persistent front and back) and migrate. Different biochemical players are

involved in the sensing stage in different systems but they often involve phosphoinositide lipids. The polarization process involves a polarity circuit comprising the RhoGTPases such as Rac, Rho and Cdc42. The interactions between these players can vary from system to system. Chemotaxis in eukaryotic systems is highly subtle as it depends not only on the underlying signalling circuits but also on the internal state of the cell and whether it is already polarized (the effects of intrinsic polarization are discussed in (Krishnan & Iglesias, 2007)), and pseudopods are being formed. This can fundamentally affect how a cell responds and migrates in response to a chemical cue. Further, signal propagation in some systems involves adaptation to homogeneous signals, whereas in other systems (eg neutrophils) homogeneous stimulation can lead to spontaneous polarization.

We examined possible design principles involving attractive and repulsive signalling in light of the above complexities. We used qualitatively simplified, rather than detailed mechanistic models. This is because we seek to distill basic qualitative insights regarding the issues under consideration. Building detailed mechanistic models would entail incorporating a lot of details, and contending with gaps in the mechanistic understanding which are tangential to and distracting from the focus of the work. Overall, the models which we employ (and combinations thereof) compactly encapsulate different possible qualitative signalling scenarios, and allow us to draw appropriate conclusions therefrom. The signalling characteristics we examined are simple feedforward local regulation (the simplest signal propagation), adaptive signalling and spontaneous polarization. All these kinds of behaviour are observed in different eukaryotic systems. In the case of adaptive signalling, the adaptive “layer” occurs in the sensing stage itself, while spontaneous polarization usually involves downstream signalling entities such as Rho GTPases. We examine these different signal propagation possibilities downstream of the individual configurations (Fig. 13). In addition to providing basic qualitative insight, our models provide a framework for also understanding and appreciating any additional complexity which occurs in the signalling network wiring of actual systems.

Polarity switch. We note that cells may exhibit attractive and repulsive behaviour to either the same or different chemicals. Further this may depend either on the signal strength or the internal state of the cell (or external medium). The first case we examined was the polarity switch—this is especially appropriate for considering attraction and repulsion to different chemicals. We found that by having a single polarity switch, an overall reversal in the response could be obtained. However the main insight here is that repellent and attractant would be regulating particular entities (in absolute concentration) in opposite directions of basal levels. This means in particular than any downstream (monostable) thresholds which were triggered by one of the chemicals, would be inaccessible for the opposite case. Such a scenario may

be however appropriate for signal transduction in already polarized cells (Arriemerlou & Meyer, 2005), where an existing pseudopod/front may simply need to be biased appropriately to guide cell motion. This further suggests that a simple polarity switch, for its functioning, may need additional features relevant to the intrinsic state of the cell for its effective functioning (and also perhaps that this repulsive response may be actually utilized or needed by the cell only when it is polarized and moving). In direct contrast, downstream adaptive signalling actually enhances the role of a polarity switch, by resetting the baseline to exactly nullify the effect mentioned above.

If a single polarity switch occurs upstream of a spontaneous polarization module, then the analysis makes a direct non-trivial prediction: if a homogeneous stimulus of chemoattractant results in spontaneous polarization, then a homogeneous stimulus of chemorepellent will not. Furthermore, if a cell spontaneously polarizes in response to a homogeneous dose of chemoattractant, this can be reversed by a sufficiently high homogeneous dose of chemorepellent, resulting in an unpolarized cell. Thus if these predictions are invalidated experimentally this means that either there is no simple single polarity switch, or that the spontaneous polarization circuit is more complex (for example having bidirectional instability thresholds, none of which have been considered in modelling thus far). It should be emphasized that while a representative module was used in our analysis the conclusion is more broadly applicable to other modules which use spontaneous symmetry breaking mechanisms. Incidentally, certain cells may spontaneously polarize in response to other signals (eg. adhesive signals) when present homogeneously, and the conclusion here is that if the chemorepellent acts on an upstream element in an exactly opposite manner to the triggering (eg adhesive) signal, then depolarization can occur for the same reasons as above.

In general it is also possible that in cells chemoattractant and chemorepellent independently switch the regulation of multiple parallel entities. This can result in a reversal in a gradient response, assuming that the entities are switched in the same way. If the regulation of the entities is itself in competition with one another, then switching their regulation is not of course guaranteed to switch the response (as this depends on the kinetics of the different regulation). Thus the observation of particular upstream entities whose regulation is switched does not necessarily imply that this is the source of the opposite response.

Competing pathways. The second scenario which we examined was the possibility that competing effects were naturally present in the gradient sensing network. Competing effects in signalling response is observed in diverse contexts in signalling, and at different levels, and is often studied in the context of hormesis. Analysis of this scenario reveals how this can naturally, under certain circumstances, reveal a reversal in the gradient response to the same signal, as the mean value of the signal is changed. Reversal of

the gradient as the mean value is increased is observed both in the neutrophil response to the chemokine Interleukin-8 as well as the T-cell response to the chemokine SDF-1 α (Tharp *et al.*, 2006; Poznansky *et al.*, 2000). It has been suggested that the gradient response reversal for high levels of chemokine may be a natural mechanism to prevent overaccumulation of cells at particular locations. If the degree of competition between pathways is increased, then such a reversed gradient response can occur at lower stimulus mean values (a prediction which can be tested in individual systems). However, having competing pathways does not guarantee the reversal of the response as signal mean value is increased. Further the effect of the presence of a spontaneous polarization circuit downstream of such a setting is that both spontaneous polarization (in homogeneous signals) and gradient reversal may be observed, and further by increasing the stimulus strength in homogeneous stimulation, a depolarization may occur. This has basic implications for the qualitative nature of the migratory response.

In our study, we notice that if a competing pathway structure exists, then it can be modulated by changes in the external or internal cellular environment so that a reversal of gradient response can be achieved even to the same gradient signal (and signal strength). Further by modulating the competing pathways it is possible to either suppress or produce or hasten the possibility of a gradient reversal as the signal mean value is increased. Noting that elements of these pathways in cells are affected by other factors and signalling we see that it is possible for cells to use the same configuration under different stages of their life cycle, other internal or external conditions, to produce very different results. Reversal of gradient response by modulating the effects of competing pathways has been studied in neural growth cones, where both modulation of pathways as well as changes in the external environment have been made. Given the apparent widespread presence of competing effects in cell signalling, it is an intriguing possibility that this may be a common occurrence in chemotactic systems.

While we have examined the effect of the competition of simple competing pathways, the results can be altered by other factors. For instance if one of the pathways has an upstream threshold, then the smooth transition from (for eg.) attractive to repulsive response can be changed to a scenario where the attractive response weakens and plateaus off for a range of concentrations before a repulsive response kicks in. Further if the signal transduction involved in the competing pathways is fundamentally different, other scenarios may be observed. A competition between adaptive and local pathways can lead to a double switching as the mean value of the signal is increased (see Appendix).

Spontaneous polarization. The other issue which has emerged involves spontaneous polarization and the fact that spontaneous polarization (in the case of a simple polarity switch) may be observed in the

case of chemoattraction or chemorepulsion but not both. The question then arises as to how a signalling network may be able to exhibit spontaneous polarization to both chemoattractants and chemorepellents. This could actually occur in different ways. One way is if chemoattractant and chemorepellent modulate different elements of the polarity circuit in a highly non-trivial way to result in spontaneous polarization for both chemoattractant and chemorepellent. A second possibility is that some global (diffusible) element upstream of the polarity circuit resets the regulation of common elements for chemoattractant and/or chemorepellent to ensure that chemorepellent may regulate the element (in mean value) in the same direction, while communicating an opposite gradient response. The other possibility is that while the signalling from each of these signals regulates the polarity circuit, they regulate different elements of the polarity circuit—an example of this could be the different isoforms of Rac or Rho which are observed in different cells. It is quite conceivable that signalling from the chemoattractant and chemorepellent may regulate different Rac isoforms, in fact, and in effect are regulating different or only partially overlapping polarity circuits. This could allow for chemoattractant and chemorepellent to both elicit their respective gradient responses as well as spontaneous polarization in homogeneous stimulation. Quite intriguingly, different Rac and Rho isoforms are observed both in the front and back of chemotaxing T-cells (Prof. Ridley, personal communication) suggesting possibly different roles for different isoforms of these RhoGTPases in some systems.

We have considered different mechanisms (polarity switch, competing pathways) upstream of adaptive and spontaneous polarization modules. Spontaneous polarization, involving Rho GTPases may be expected to be downstream of such effects, but adaptation may occur at the sensing level. Could adaptive signalling be upstream of the polarity switch/competing pathways? Adaptive signalling upstream of a polarity switch would still provide a reversal in the gradient response. Adaptive signalling upstream of competing pathways would however prevent the signal level dependent reversal in gradient response.

Our study strongly suggests a systematic effort to elucidating both attractive and repulsive responses in cells using both modelling and focussed experiments. In particular it is important to study the response of cells (both mobile and immobilized, where possible) to different stimuli (homogeneous, gradient) of both chemoattractant and chemorepellent: this includes a systematic study of the mean value and gradient strength of the stimulus and monitoring the response of components such as phosphoinositide lipids (or relevant components involved in gradient sensing), as well as the Rho GTPases. Care must be taken to ensure that cells are in essentially the same initial state. Building on this a systematic study of the effect of modulating different (especially competing) pathways biochemically would reveal important information.

If a polarity switch is suspected, it is important to systematically examine and compare temporal and spatial behaviour of downstream components to different chemoattractant and chemorepellent stimuli. Our study serves as a basis for examining different characteristic signalling responses. It also suggests that based on the results of experimental investigations suggested, particular attention be paid in modelling how gradient sensing signalling regulates the Rho GTPases. A detailed elucidation of how individual cells are wired to exhibit attractive and repulsive responses would provide invaluable insight into the different kinds of control of cell migration pathways, the manner and conditions under which the cell would exhibit each of these responses, as well as the essential constraints involved in each case. It would also provide clues as to the contexts in which these responses are needed, as well as how the capacity to exhibit one response may have been built on the other.

In conclusion we have formulated a framework, from an implicit systems perspective, for analyzing some design principles which might allow cells to exhibit attractive and repulsive response to either the same or different signals. In fact this framework would also provide insights in the case where the signals may be of different types (not necessarily chemical). Understanding this aspect of migrating cells would be a very convenient way of starting to examine how the cellular behaviour may be exploited or reversed. Noting that cells likely naturally have such capabilities through evolution, a careful exploitation of this aspect can allow for the control of directional migration even if various details of downstream signalling are not completely elucidated. This in turn could be of considerable use and set the stage for a systematic exploitation of this feature in biological, physiological, medical and synthetic contexts.

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5 Appendix

5.1 Competition between local and adaptive pathways

The discussion in the previous subsections was based on an analysis of the competition between local (simple feedforward) pathways in determining the gradient response. It also highlighted the effect of diffusion of those components. It is worth examining whether there is any essential difference if the signalling involved in either of the pathways is different from a simple local regulation. In order to examine this, we will examine the case of competition between an adaptive and a local pathway.

For simplicity, we will assume that, unless otherwise stated, all components are weakly or non-diffusible. For specificity, we will first examine the competition between an adaptive LEGI module

(adaptive module used in the text with $k_{da} = 0, k_{di} \gg 1$), and a local inhibitory module. The equation for the adaptive module is as given in the text, and the output is R^* : the activator is non-diffusible and the inhibitor is highly diffusible. The regulation of the local regulatory module is as in previous sections and the output is B^* . Both these components regulate the conversion of a response element from its inactive to active form, and it is assumed for simplicity that the corresponding rate constants are the same. Thus in order to determine the nature of the gradient response, it suffices to examine the behaviour of $R^* + B^*$.

The steady state response $R^* + B^*$ may be written (for an input signal $S(\theta) = a + b\cos\theta$) as

$$R^* + B^* = \frac{(k_a k_{-i}/k_i k_{-a})(1 + (b/a)\cos\theta)}{k_r/k_f + (k_a k_{-i}/k_i k_{-a})(1 + (b/a)\cos\theta)} R_{tot} + \frac{k_{fb}}{k_{fb} + k_b(a + b\cos\theta)} B_{tot} \quad (24)$$

In the above the adaptive module has a local excitation and highly diffusible inhibitory component. The steady state response of the adaptive and local module has been used in obtaining the above expression.

The net gradient response depends on the relative quantities R_{tot}, B_{tot} , the total amount of active and inactive response elements for each of the two pathways. Note that above, the adaptive module has $R + R^* = R_{tot}$, which is reflected in the expression above. In an exactly analogous way we can examine the competition between a repulsive adaptive module (with $k_{di} = 0, k_{da} \gg 1$) and a local attractive module. In this case we have

$$R^* + B^* = \frac{(k_a k_{-i}/k_i k_{-a})}{k_r/k_f(1 + (b/a)\cos\theta) + (k_a k_{-i}/k_i k_{-a})} R_{tot} + \frac{k_b(a + b\cos\theta)}{k_{bb} + k_b(a + b\cos\theta)} B_{tot} \quad (25)$$

Simulations showing the net response to competing adaptive and local modules are seen in Fig 14. This is done for a repulsive adaptive and a local attractive pathway. Here the effect of the relative strengths of the two pathways are examined. We also analyzed the response to different gradient signals, keeping the relative gradient fixed. In Fig. 14(b), we examined a case where the mean value of a signal was increased again keeping the relative gradient strength fixed. For the choice of parameters, this always revealed a repulsive response. In other words the adaptive pathway always wins. Fig. 14(a) shows another aspect of the interaction of adaptive and local pathways. Here, as the mean value of the signal increases (keeping the relative gradient fixed), we see that for relatively low mean values, the response is repulsive, for higher mean values, the response is attractive and for still higher mean values the response is again repulsive. This behaviour may be understood as follows. For very low mean values of signals, the adaptive pathway dominates as it depends only on the relative gradient and not on the mean value of the signal, while the local pathway hardly registers the presence of the signal. For higher mean values of the signal, the local pathway contributes, and for these parameter values effectively defeats the contribution of the adaptive

module. Finally for still higher mean values of the signal, the local pathway saturates and the adaptive pathway wins (note that the adaptive pathway does't include any saturation effects). This simple study shows how the competition between pathways of different kinds can lead to quite subtle net responses, which may be different from competition of two local signalling pathways. While this double switching was observed for the case where the relative gradient was kept fixed, a similar kind of double switching is seen where the mean value of the signal is increased keeping the (absolute) gradient fixed (results not shown). The qualitatively same kind of double switching behaviour can also be seen in the case where the adaptive pathway is attractive and the local pathway is repulsive. In this case the gradient response can change from attractive to repulsive back to attractive when (for instance) the mean value of the signal is changed keeping the relative gradient fixed.

Some further analytical insight into the kind of above behaviour may be obtained by examining the above expressions. In particular for the case of attractive adaptive regulation (LEGI) and local repulsive regulation, the response may be written as

$$R^* + B^* = R_{tot} - R_{tot} \frac{k_r/k_f}{k_r/k_f + (k_a k_{-i}/k_i k_{-a})(1 + (b/a)\cos\theta)} + \frac{k_{fb}}{k_{fb} + k_b(a + b\cos\theta)} B_{tot} \quad (26)$$

It thus suffices to examine the last two terms to determine the spatial response of the solution.

The net response has the functional form

$$F(\theta) = \frac{-A_1}{a_1 + b_1 \cos\theta} + \frac{A_2}{a_2 + b_2 \cos\theta} \quad (27)$$

where $A_1, A_2, a_1, a_2, b_1, b_2$ are all constants. The maxima/minima are obtained by setting the derivative with respect to angle to zero. This results in

$$\frac{-A_1 b_1 \sin\theta}{(a_1 + b_1 \cos\theta)^2} + \frac{A_2 b_2 \cos\theta}{(a_2 + b_2 \cos\theta)^2} \quad (28)$$

This results in $\theta = 0, \pi$ and also possibly an intermediate extremum. We will particularly focus on $\theta = 0, \pi$ as solutions. To determine whether the extrema $\theta = 0, \pi$ are maxima/minima, we need to find the second derivative of the above expression. The second derivative of the above expression at $\theta = 0, \pi$ is given by

$$F''(\theta) = \frac{-A_1 b_1 \cos\theta}{(a_1 + b_1 \cos\theta)^2} + \frac{A_2 b_2 \cos\theta}{(a_2 + b_2 \cos\theta)^2} \quad (29)$$

Note that in the above, other terms which are zero at these locations are not included. In particular the second derivative at $\theta = 0, \pi$ is given by

$$\begin{aligned} F''(0) &= \frac{A_2 b_2}{(a_2 + b_2)^2} - \frac{A_1 b_1}{(a_1 + b_1)^2} \\ F''(\pi) &= \frac{-A_2 b_2}{(a_2 - b_2)^2} + \frac{A_1 b_1}{(a_1 - b_1)^2} \end{aligned} \quad (30)$$

Now $\theta = 0$ corresponds to a maximum if

$$\frac{A_1 b_1}{(a_1 + b_1)^2} > \frac{A_2 b_2}{(a_2 + b_2)^2} \quad (31)$$

and likewise $\theta = \pi$ corresponds to a maximum if

$$\frac{A_1 b_1}{(a_1 - b_1)^2} < \frac{A_2 b_2}{(a_2 - b_2)^2} \quad (32)$$

From the above it is easy to determine whether each of these locations corresponds to a maximum or a minimum. In particular the conditions above can be written as

$$\begin{aligned} \frac{A_1}{A_2} &> \frac{b_2(a_1 + b_1)^2}{b_1(a_2 + b_2)^2} \\ \frac{A_1}{A_2} &< \frac{b_2(a_1 - b_1)^2}{b_1(a_2 - b_2)^2} \end{aligned} \quad (33)$$

The point to note here is that these conditions are not mutually exclusive. Some analysis reveals that if $b_1/a_1 > b_2/a_2$ then $\theta = 0$ is a maximum and $\theta = \pi$ is a minimum. Under other conditions it is possible for an intermediate maximum to occur.

Now using these expressions, and inserting the expressions above for the response, reveals when $\theta = 0$ corresponds to a maximum and a minimum. In fact the analysis reveals the kind of switching which is observed in the simulations. A very similar analysis can be performed for the case of repulsive adaptive and local attractive pathways. The only difference is that the net response has the form

$$F(\theta) = \frac{A_1}{a_1 + b_1 \cos \theta} + \frac{-A_2}{a_2 + b_2 \cos \theta} \quad (34)$$

and a very similar analysis can be performed.

In summary, by considering the competing between an adaptive and a local pathway it is possible to see a more complex double switching as the signal mean value is increased.

5.2 Switching response in temporal sensing

In the text we examined different potential design principles which allowed a given cell to exhibit both attractive and repulsive sensing. The analysis was based on spatial sensing mechanisms. One of the settings we focussed on was whether the opposite regulation of an upstream reaction by attractant and repellent could be the basis of the opposite nature of the response.

In this section, we examine this in the case of a purely temporal sensing chemotactic mechanism, which involves adaptation: the gradient sensing mechanism in E.coli. This is a widely studied system,

which exhibits adaptive sensing over a wide range of concentrations. We focus on a simplified description of the signalling, based on a reduction of the well-known Barkai Leibler model for sensory transduction (Barkai & Leibler, 1997). The model has three components active methylated receptors X_m^* (the output), inactive methylated receptors X_m and demethylated receptors X_0 (Alon, 2006). A chemoattractant deactivates the methylated receptors converting it into inactive methylated receptors. It is assumed that demethylation occurs only of active methylated receptors (via the enzyme CheB) and demethylated receptors can be methylated by the enzyme CheR, a reaction assumed to follow zeroth order kinetics. An increase in the output tends to increase the probability of the bacterium to tumble (and change direction).

The equations for the model are given below.

$$\begin{aligned} dX_m^*/dt &= -k_b S X_m^* + k_f X_m - k_1 CheB X_m^* / (k_{m1} + X_m^*) \\ dX_m/dt &= k_b S X_m^* - k_f X_m + k_1 CheR X_0 / (k_{m2} + X_0) \end{aligned} \quad (35)$$

If the enzymatic reaction involving CheR is acting as a zeroth order reaction, then the dependence on X_0 drops out. Adding the above two equations, and examining this at steady state immediately reveals that the steady state response X_m^* is independent of the signal.

It is worth examining how a chemorepellent would work in the similar situation. If a chemorepellent mediates the opposite reaction as the chemoattractant above (the equivalent of a polarity switch), then we immediately see by repeating the analysis above that the response still exhibits exact adaptation with the opposite temporal response. This indicates that the mediation of the opposite reaction can give rise to the desired opposite response, preserving the adaptation property.

Another point to be made is that the chemorepellent may be involved in the activation of different methylated receptors (from the chemoattractant), which involve a very similar network structure and dynamics. In this case, while an adaptive opposite response may be observed, the repellent may be involved in regulating the reverse reaction for a different methylated receptor pair (active/inactive). It is of course possible that a repellent may be regulating the opposite reaction for a different methylated receptor pair, which does not have the same kind of dynamics/methylation or even show adaptation.

Fig. 1. Schematic of modules Schematic diagrams of Local, Adaptive and Spontaneous Polarization modules. A. The local module contains an input signal labelled S that regulates the response R^* . R^* is capable of diffusing. B. The adaptive module consists of an input signal that regulates an activator A and an inhibitor I which in turn regulate the response element R^* . A and I may or may not diffuse. C. In the spontaneous polarization module the input signal S regulates two activators u_2 and u_3 .

These activators inhibit one another and upregulate the production of an inhibitor u_1 . u_1 inhibits both u_2 and u_3 and diffuses as well (see text for details).

Fig. 2. Response of local and adaptive modules A. For the local module, an attractive response (solid line) is seen when the external signal upregulates the response and repulsive (solid line with circles), when it is downregulated by the external signal. Here and in other diagrams, unless mentioned, the signal is $S = 1 + 0.4\cos\theta$, with a maximum at $\theta = 0$. B. The adaptive module response is attractive (solid line) when $k_{di}/k_{-i} - k_{da}/k_{-a}$ is positive. A repulsive response (solid line with circles) is achieved when this quantity is negative. Parameter values: local module $k_f = 2.0; k_r = 1.0; k = 1.0; k_d = 0.0$. Adaptive module: attractive $k_a = 1.0; k_{-a} = 1.0; k_i = 2.0; k_{-i} = 2.0; k_f = 1.0; k_r = 1.0; k_{da} = 0.0; k_{di} = 10.0$. For the repulsive response in the adaptive module, diffusivities of activator and inhibitor are interchanged.

Fig. 3 Response of the spontaneous polarization module Attractive and Repulsive responses from the spontaneous polarization module. Attractive responses (A,C): The signal is $S = 1 + 0.05\cos\theta$ (A) and $S = 1 + 0.4\cos\theta$ (C). The inhibitor (solid line) and the two activators u_2 (dotted line) and u_3 (solid line with circles) are shown. Attractive responses are obtained for these set of parameter values $a_{12} = 4.0; a_{13} = 1/3; a_{21} = 3/2; a_{22} = 1/2; a_{23} = 1/2; a_{31} = 1.0; a_{32} = 2.0; a_{33} = 1.0; \rho_2 = 2.0, \rho_3 = 1.0$. The activator u_2 (frontness pathway) is in phase with the signal. Repulsive responses (B,D): Again the same signals $S = 1 + 0.05\cos\theta$ (B) and $S = 1 + 0.4\cos\theta$ (D) are employed. Repulsive responses are obtained for the following set of parameter values: $a_{12} = 1.0/2.0; a_{13} = 4.0; a_{21} = 1.0; a_{22} = 1.0; a_{23} = 2.0; a_{31} = 3.0/2.0; a_{32} = 1.0/2.0; a_{33} = 1.0/2.0; r_2 = 1.0; r_3 = 2.0$; The activator u_3 (backness pathway) is now in phase with the signal.

Fig. 4: Schematic of modules with extra element The figure shows the three modules with an extra element S^* which may be activated or inhibited by the attractant and repellent respectively. Only the scenarios corresponding to the activation case are shown.

Fig. 5: Switching of local and adaptive modules by altering the behaviour of upstream components Switching from an Attractive to a Repulsive response by regulating an upstream component. A. In the local module the attractive (solid line) and the repulsive (dotted line) is achieved by positively or negatively regulating the upstream component by the external signal, respectively. If the kinetic constant k_{f1} is decreased ($=0.1$) then the repulsive response (solid line with circles) is obtained that

is overall reduced. Parameters are $k_{f1} = 2, k_{r1} = 1, k_1 = 1$. B. In the adaptive module the attractive (solid line) and the repulsive (dotted line) is achieved by positively or negatively regulating the upstream component by the external signal respectively. We notice that adaptive signalling downstream of a polarity switch, provides the opposite biasing without decreasing the overall response everywhere. Parameters are $k_{f1} = 1.0, k_{r1} = 1, k_1 = 1$.

Fig. 6: Switching of spontaneous polarization element by altering regulation of upstream components

Switching to a repulsive response from an attractive one in the spontaneous polarization module by regulating the upstream component. The upstream component (+sign), inhibitor u_1 (solid line) and the two activators u_2 (dotted line) and u_3 (solid line with circles) are shown. A. The attractive and B. the repulsive response is achieved by locally positively and locally negatively regulating the upstream component by the external signal, respectively, as is seen from the location of the frontness component u_2 . Note that in B, while a repulsive response is achieved, it is rather weak without a clear and sharp separation of frontness and backness components. C. By altering the regulation of the upstream component to include dual activation (global) and inhibition (local) it is possible to obtain a repulsive response which makes use of the symmetry breaking mechanism that is the core of the attractive mechanism. The signal is $2 + 0.4\cos\theta$. Parameters for (a) and (b) are $k_{f1} = 1, k_{r1} = 1, k_1 = 1$ For (c) $k_{f1} = 1, k_1 = 1, k_{r1} = 1, k_{da} = 10.0$.

Fig.7 Schematic diagram of competing pathways A schematic diagram of competing pathways with the signal mediating the forward regulation of A and the backward regulation of B are shown. A and B have additive effects in regulating a response element.

Fig. 8 Parallel local regulation of pathways This figure depicts certain qualitative aspects of the competition between pathways. (a) When both components A and B are positively regulated by the signal, an attractive response (solid line) is achieved. When both A and B are negatively regulated by the external signal a repulsive response (dotted line) is observed. (b) When A is positively regulated by the signal and B is negatively regulated by the signal an attractive response is achieved (solid line). And when A is negatively regulated by the signal and B is positively regulated by the signal a repulsive response is seen (dotted line). Parameter values for (a) are:

$k_a = 2.0; k_{fa} = 0; k_{ba} = 1.0; k_b = 1.0; k_{fb} = 0.0; k_{bb} = 1.0$ for the parallel positive regulation and $k_a = 1.0; k_{fa} = 2.0; k_{ba} = 0.0; k_b = 1.0; k_{fb} = 1.0; k_{bb} = 0.0$ for the parallel negative regulation. In both cases $A_{tot} = 1; B_{tot} = 0.5, k_f = k_r = 1.0; R_{tot} = 3.0$. Parameter values for (b) are

$k_a = 2.0; k_{ba} = 1.0; k_{fb} = 1.0; k_b = 1.0$ for positive regulation of A and negative regulation of B and $k_{fa} = 2.0; k_a = 1.0; k_b = 1.0; k_{bb} = 1.0$ for the positive regulation of B and negative regulation of A. $k_f = 1.0; k_r = 1.0; B_{tot} = 0.5; A_{tot} = 1.0; R_{tot} = 3.0$. Any other constants are zero.

Fig. 9 Competition of pathways (a) A is positively regulated by the signal and B is negatively regulated by the signal. When the total amount of A is much greater than B (1.4 and 0.3, respectively) (solid line) an attractive response is achieved. However when the total amount of B is greater than A (0.7 and 0.6, respectively) (dotted line) then a repulsive response is achieved. (b) A is negatively regulated by the signal and B is positively regulated by the signal. In this scenario, the net sensing response is qualitatively the opposite of that of (a). Parameter values for (a) are $k_a = 2.0; k_{ba} = 1.0; k_{fb} = 1.0; k_b = 1.0; k_f = 1.0; k_r = 1.0$. Parameter values for (b) are $k_{fa} = 2.0, k_a = 1.0, k_b = 1.0, k_{bb} = 1.0, k_f = 1.0, k_r = 1.0$.

Fig. 10 The variation of response to homogeneous signals for the case of competing pathways In this case A is activated and B is inhibited by the signal. Different scenarios depicting the variation of response to the signal value (dose-response curve) are shown. For simplicity the total upstream signal $A + B$ regulating the response is plotted. (a) Response is a monotonically increasing function of signal value. (b) Response monotonically decreases (c) Response monotonically increases and then decreases. (d) Response monotonically decreases and then increases. In all these cases the slope of the curve provides information about the gradient response for weak gradients superimposed upon a mean value of signal corresponding to that location of the curve, in the case where the pathways are non-diffusible. Parameter values are: $k_a = 2.0; k_{ba} = 1.0; k_{fb} = 1.0; k_b = 1.0$; and (a) $A_{tot} = 0.7, B_{tot} = 0.3$ (b) $A_{tot} = 0.3, B_{tot} = 0.7$ (c) $A_{tot} = 0.5, B_{tot} = 0.5$. For (d) $A_{tot} = 0.5, B_{tot} = 0.5, k_a = 1.0; k_{ba} = 1.0; k_{fb} = 1.0; k_b = 2.0$.

Fig. 11 The gradient response for the case of competing local pathways This figure shows the qualitative effect of mean value of signal reversing the gradient response, and also the effects of the competing pathways. (a) A is positively regulated by the signal and B is negatively regulated by the signal and $A_{tot} = B_{tot}$. The response for the external signal $S = 0.5 + 0.1\text{Cos}\theta$ (solid line) and $1.0 + 0.2\text{Cos}\theta$ (dotted line) is shown. Here the mean value is changed keeping the relative gradient fixed. A reversal in the nature of the gradient response is observed. A similar qualitative effect is observed if the mean value is changed keeping the gradient strength fixed. Parameters are the same as Fig. 10 (a), except that $A_{tot} = B_{tot} = 0.5$. (b) B is positively regulated by the signal and A is

negatively regulated by the signal and $A_{tot} = B_{tot}$. The response for the external signal $S = 1 + 0.4\cos\theta$ (solid line) and $S = 2.0 + 0.4\cos\theta$ (dotted line) is shown. In this case, the mean value of the signal is varied keeping the gradient strength fixed. Again we see a switching in response. Parameters are the same as in Figure 10 (part (a)) except $k_{ba} = 2, A_{tot} = B_{tot} = 0.5$. (c) The effect of varying the strengths of the competing pathways for a fixed signal is considered. A is positively regulated and B is negatively regulated by the signal $S = 1 + 0.2\cos\theta$. Here the parameters A_{tot} and B_{tot} are varied and the qualitative effect of this change on the response is observed. A reversal in the gradient response is observed when we compare the case of $A_{tot} = 1.0$ and $B_{tot} = 2.0$ (solid line, repulsive response) and $B_{tot} = 1.0$ and $A_{tot} = 2.0$ (solid line with circles, attractive response). The parameters are $k_a = 1.0; k_{ba} = 1.0; k_{fb} = 1.0; k_b = 1.0, R_{tot} = 10.0$.

Fig. 12 The effect of spontaneous polarization downstream of competing pathways In this figure we consider the case of competing pathways A (positively) and B (negatively) regulated by the signal: (parameters for A and B are the same as Fig. 10(c) except that $k_f = 0.01, R_{tot} = 115$. The competing pathways regulate a response (R^*), which serves as the input to the spontaneous polarization module. The response of the combined system to three different gradient signals is shown: (a) $S = 0.3 + 0.03\cos\theta$: the response is indicative of an attractive response (frontness component u_2 (dotted line) co-localized with the maximum of the signal) which makes use of the symmetry breaking mechanism: note that the polarization of frontness and backness are much stronger than the external signal. (b) $S = 5 + 0.03\cos\theta$: here the response is representative of a repulsive response, which is also sharply polarized (and making use of the instability mechanism). (c) $S = 30 + 0.03\cos\theta$: here the response is very weakly repulsive for the same gradient strength, and there is no sharp polarization of frontness and backness components. (d) A schematic of the dose-response curve for the competing non-diffusible pathways, labelling the implication of downstream spontaneous polarization for the overall gradient response. The slope of the curve indicates the nature of the gradient response, while its position relative to the spontaneous polarization line indicates whether that mechanism is employed in the gradient response.

Fig. 13 A tabular summary of different switching mechanisms This figure compactly summarizes the different kinds of upstream mechanisms, the different downstream signalling effects, and the different kinds of switching which can result from any combination thereof. CA and CR refer to chemoattractant and chemorepellent.

Fig. 14 The competition of local and adaptive pathways This figure considers the competition between an adaptive (repulsive) and a local (attractive) pathway. A corresponds to the adaptive pathway and B corresponds to the local pathway. Signals of fixed relative gradient of the form $S = \alpha(1 + 0.4\cos\theta)$ were applied. (a) $A_{tot} = 0.3, B_{tot} = 0.7, \alpha = 0.5$ (solid line) indicates a repulsive response, $\alpha = 5$ (dotted line) corresponds to an attractive response and $\alpha = 10$ (solid line with circles) corresponds again to a repulsive response. This figure qualitatively shows the double reversal of gradient sensing response. (b) Here $A_{tot} = 0.7, B_{tot} = 0.3$ and for these same three signals, the response is always repulsive indicating that the adaptive pathway always dominates.

$$k_a = 1.0; k_{-a} = 1.0; k_{da} = 10.0; k_i = 2.0; k_{-i} = 2.0; k_r = 1.0; k_{-r} = 1.0; k_{fb} = 1.0; k_{bb} = 1.0; k_{rb} = 1.0; k_f = 1.0; R_{tot} = 1.0; k_b = 1.0;$$

Figure 1

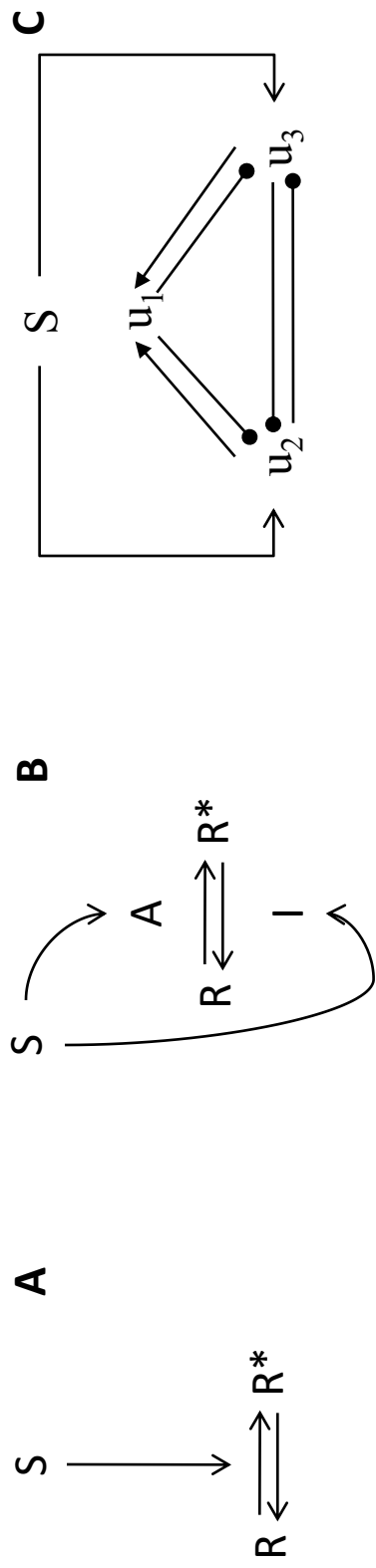


Fig.1

Figure 2

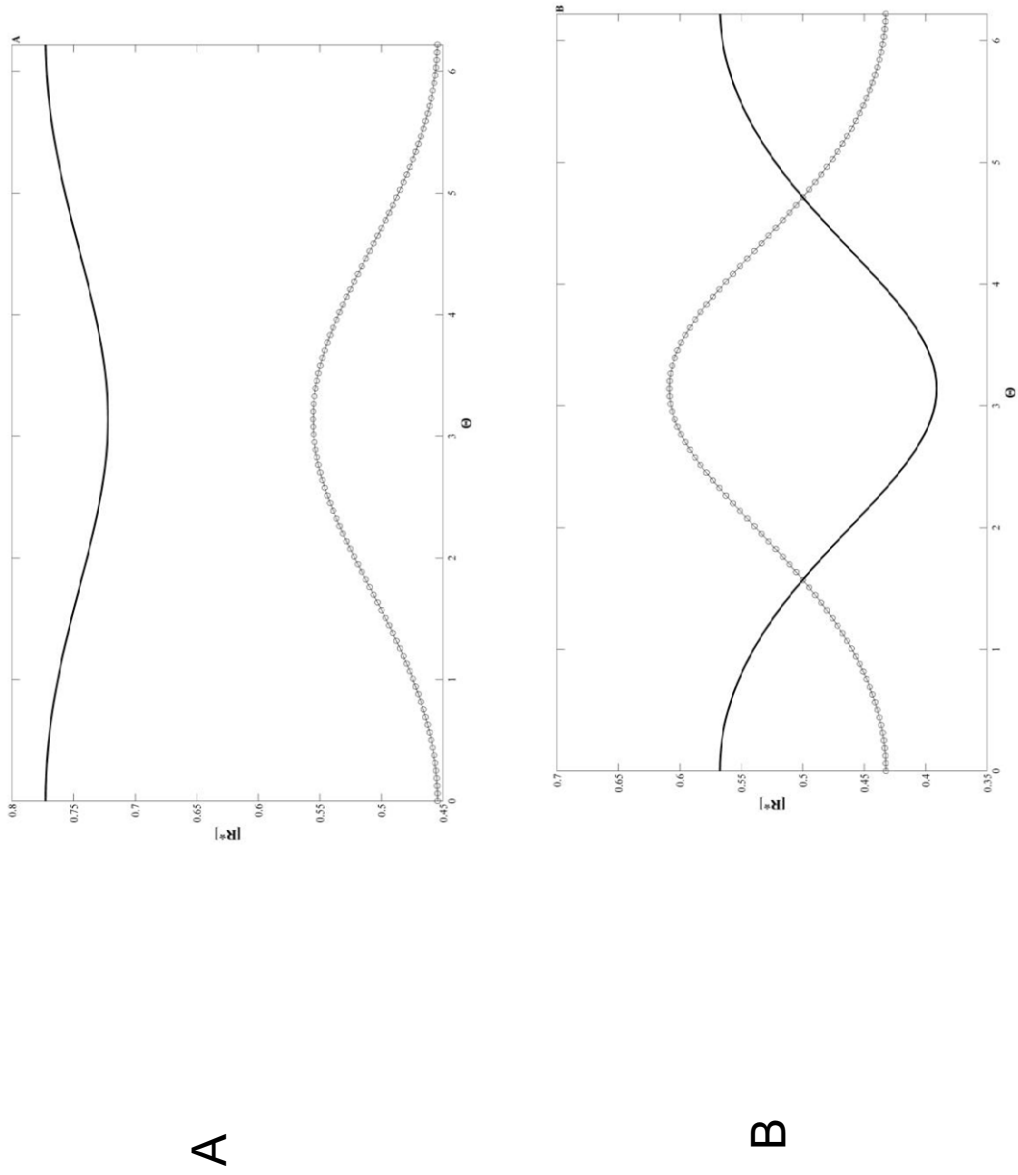


Fig.2

Figure 3

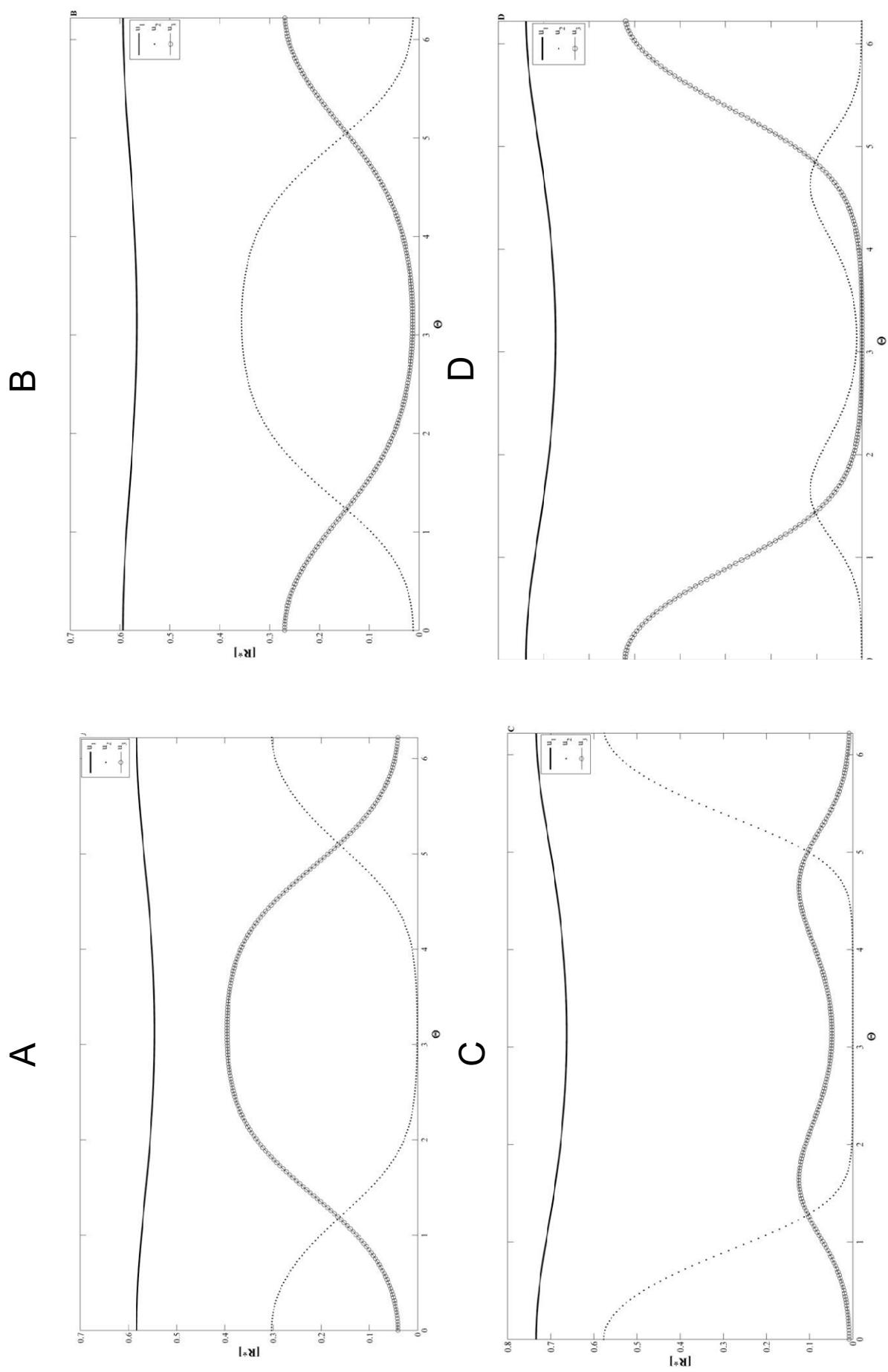


Fig.3

Figure 4

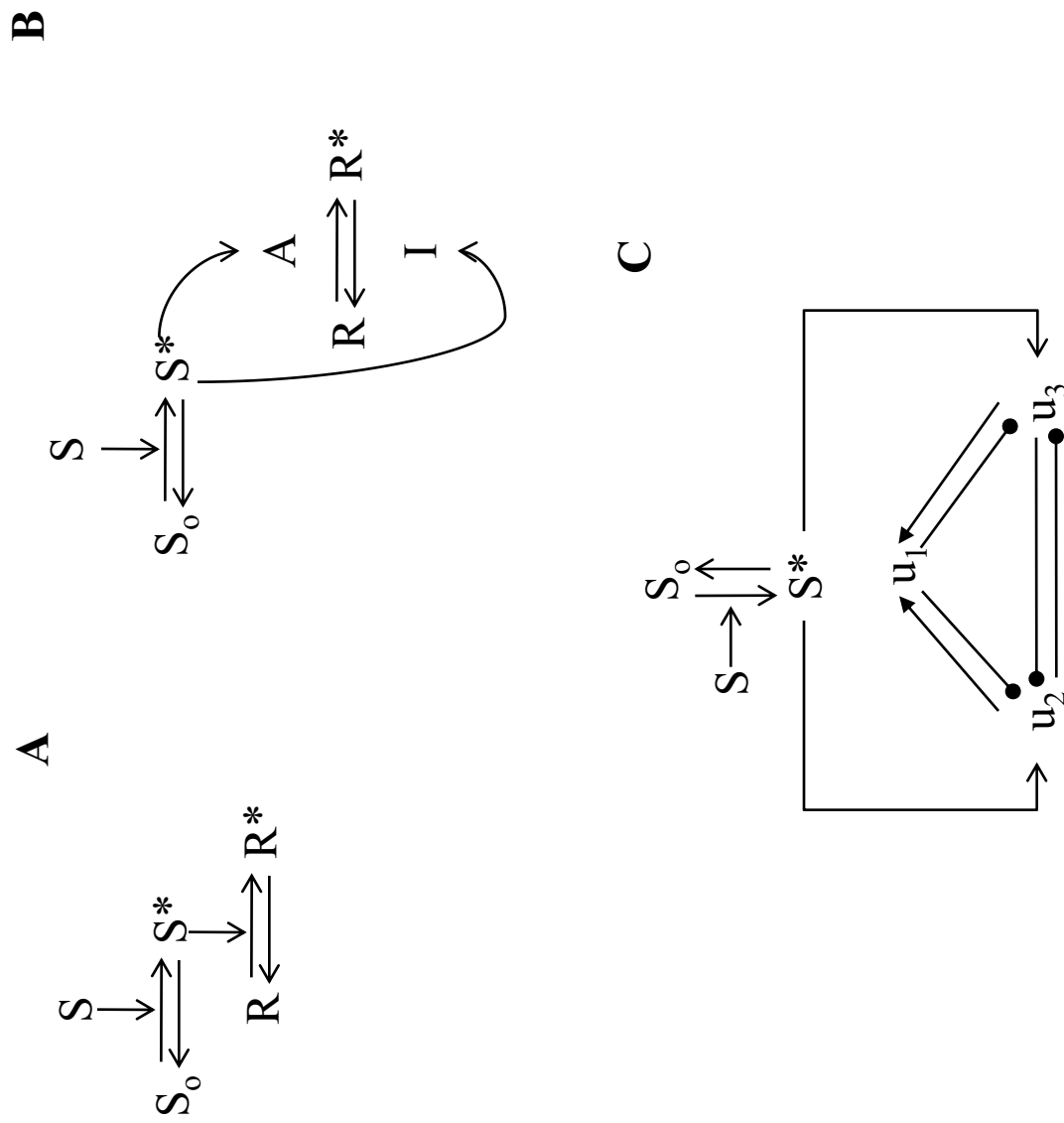


Fig.4

Figure 5

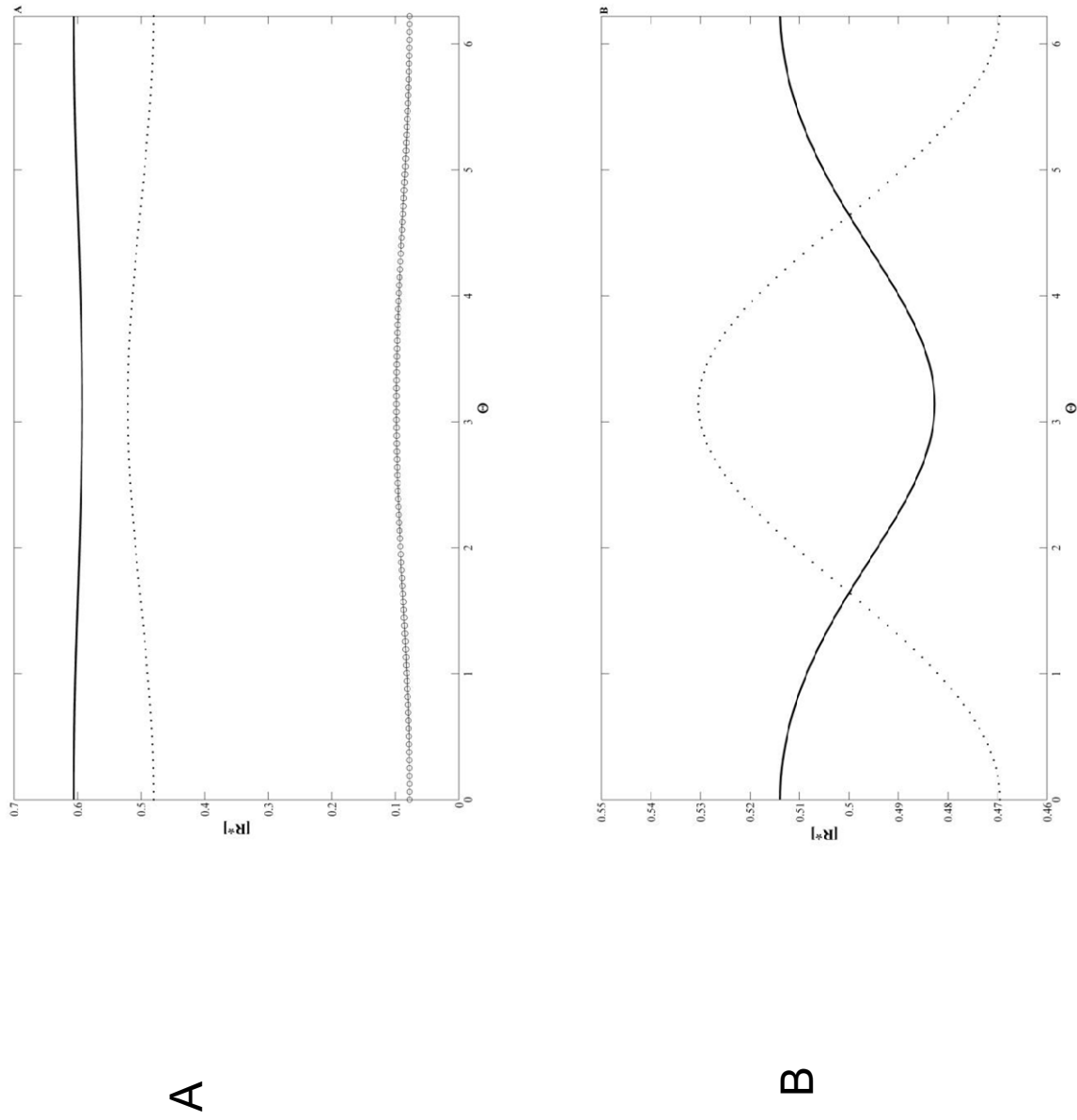


Fig.5

Figure 6

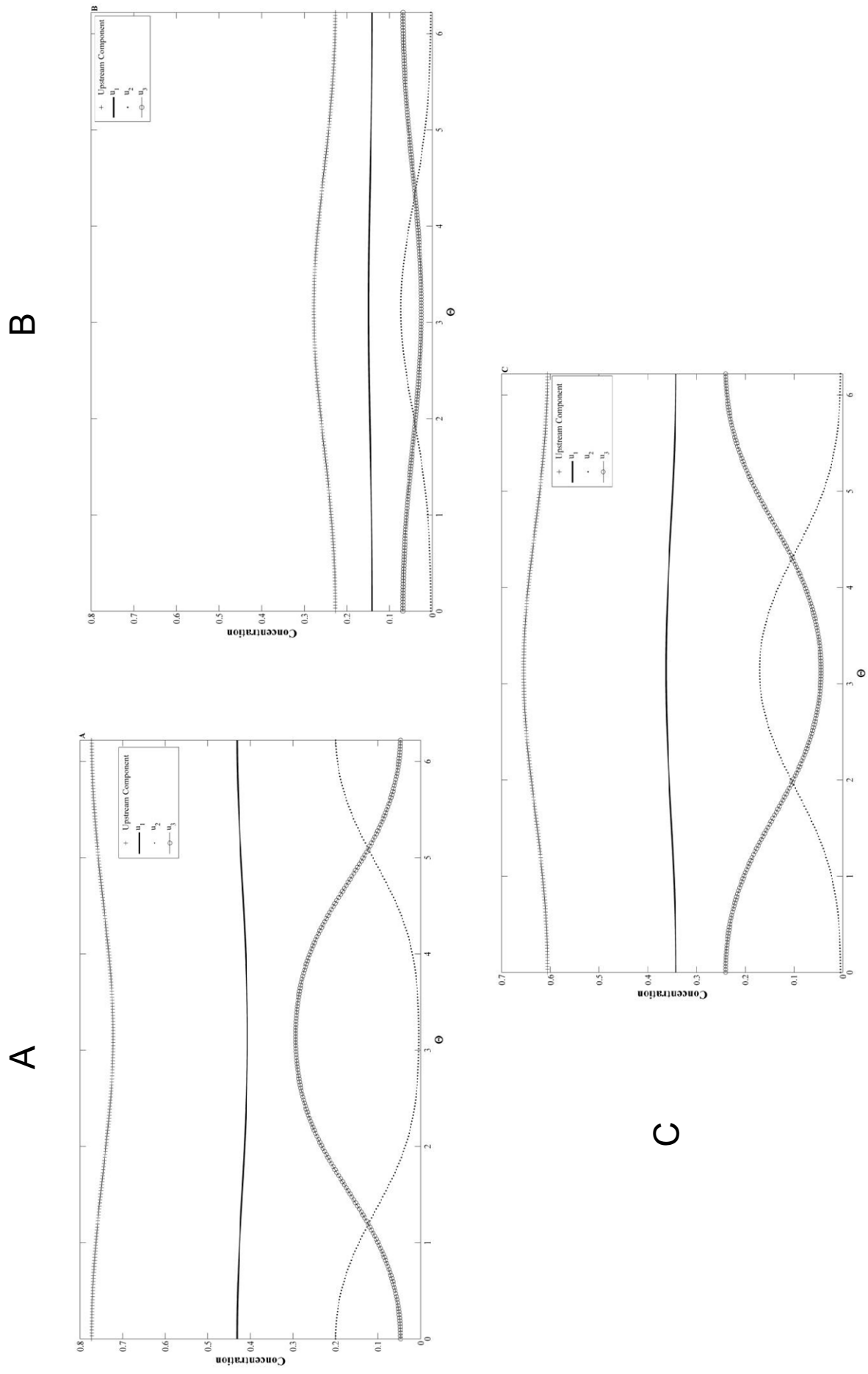


Fig.6

Figure 7

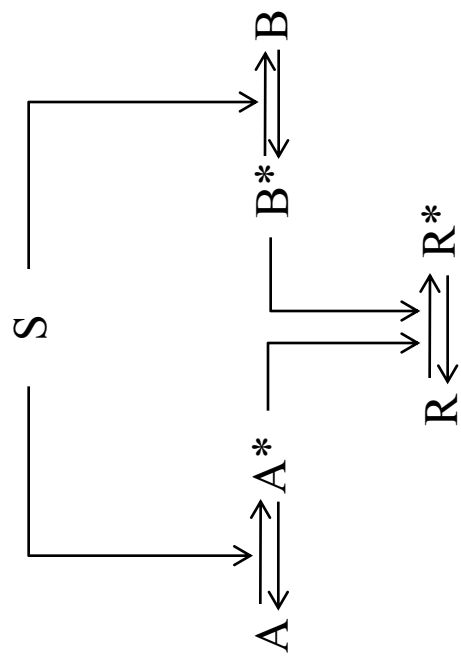


Fig.7

Figure 8

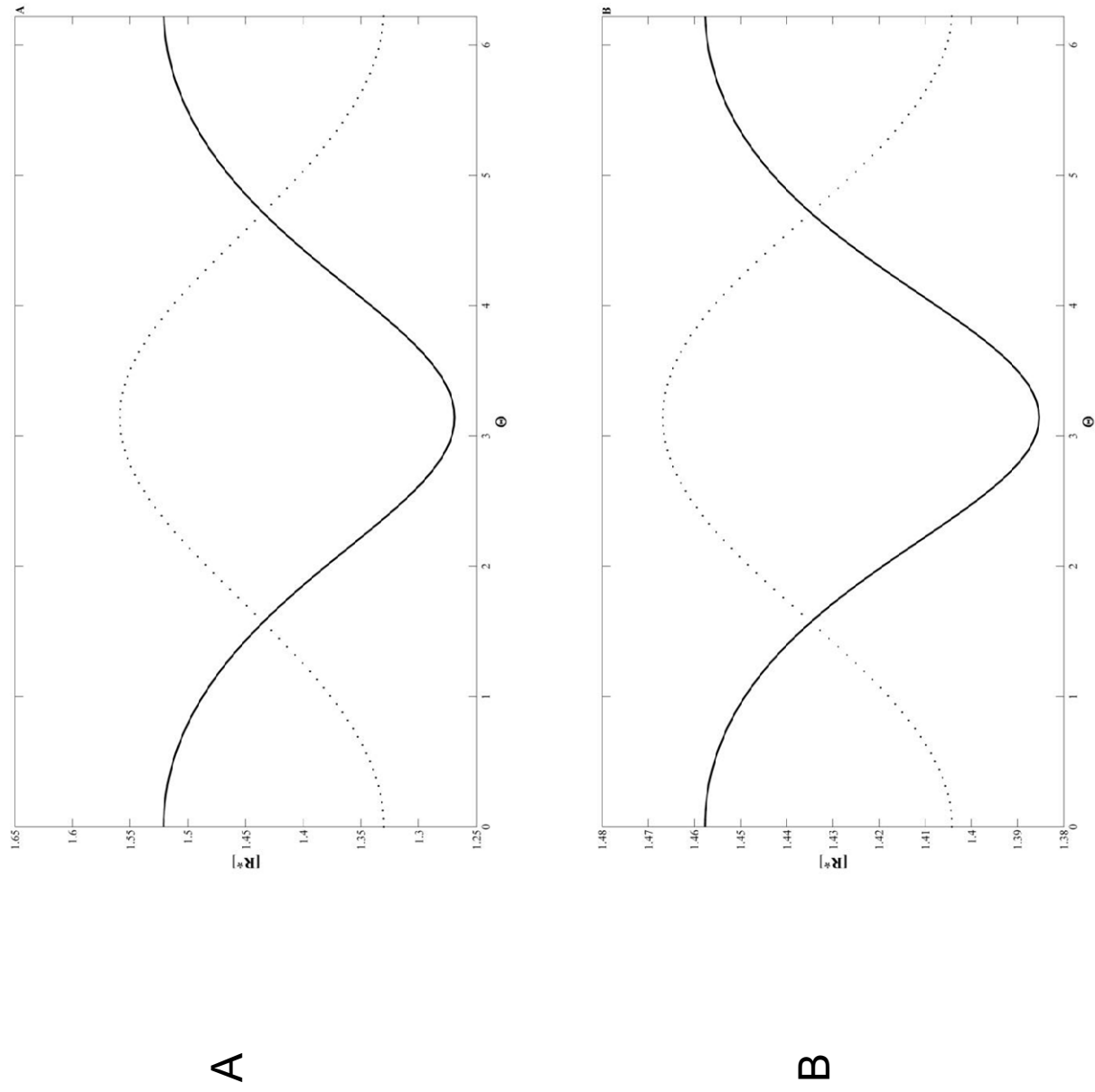


Fig.8

Figure 9

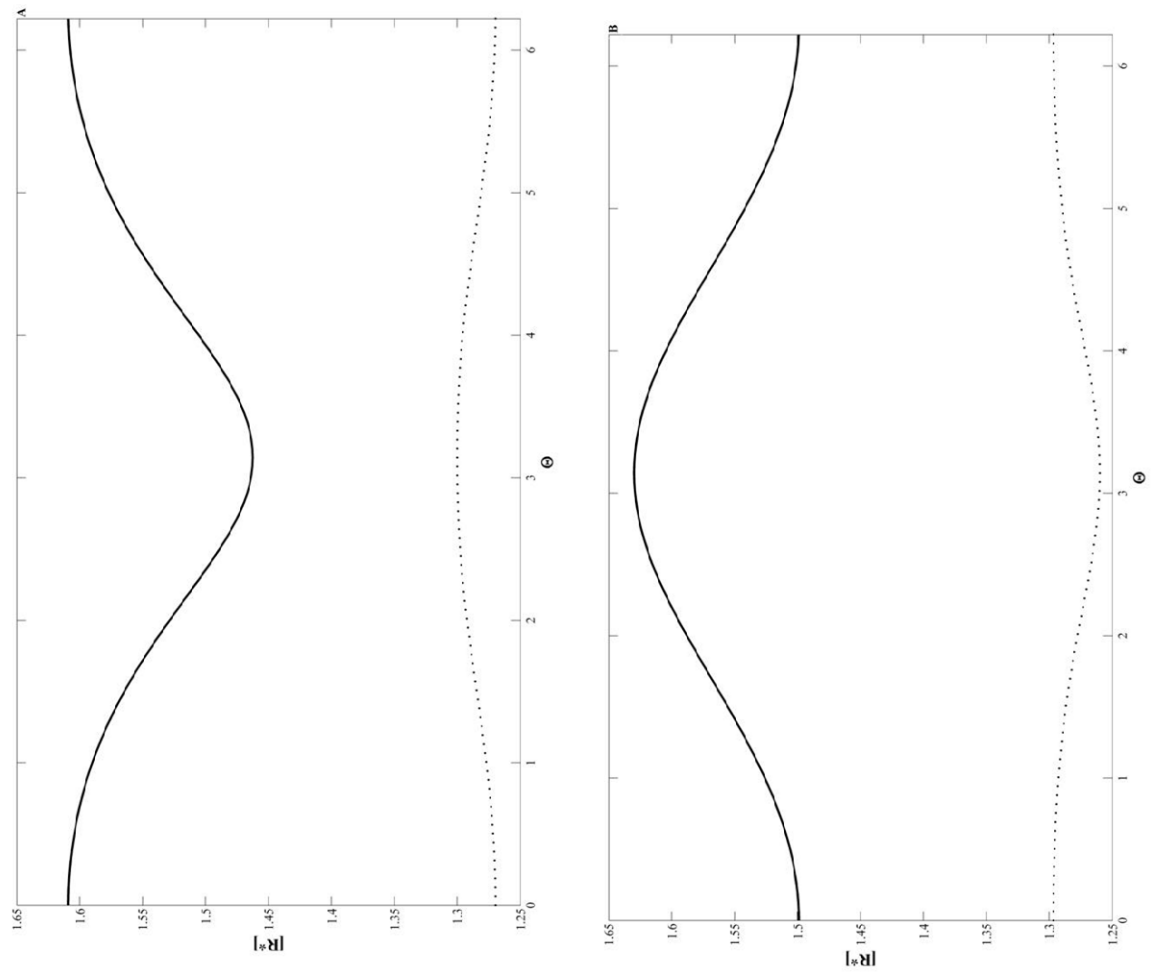


Fig.9

Figure 10

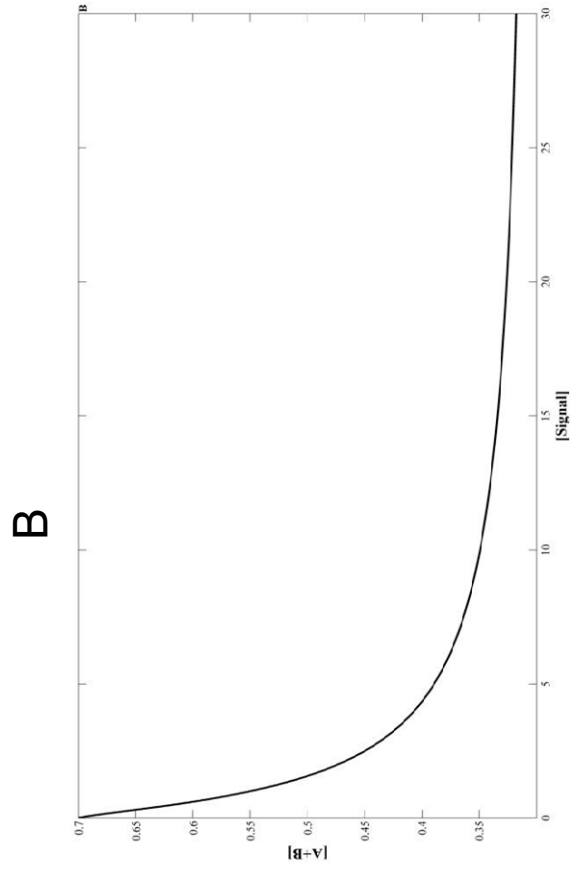
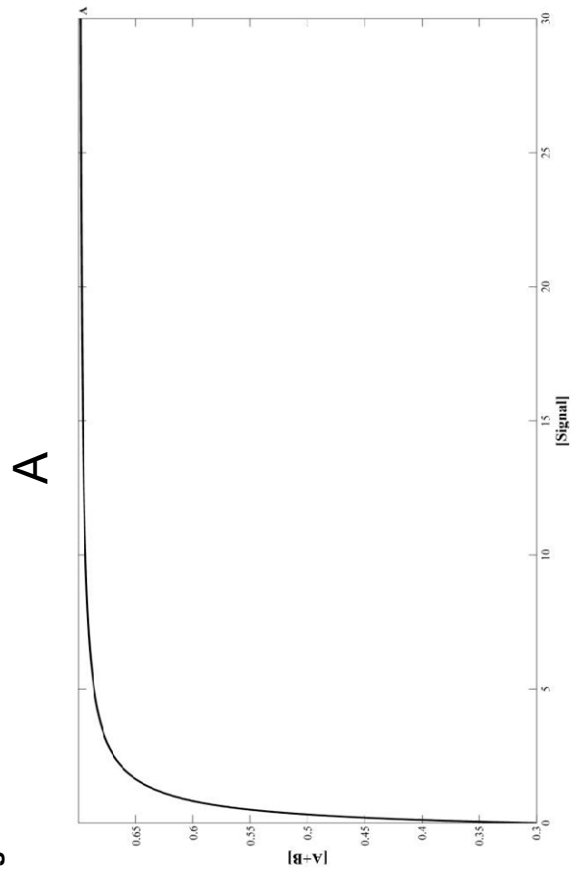
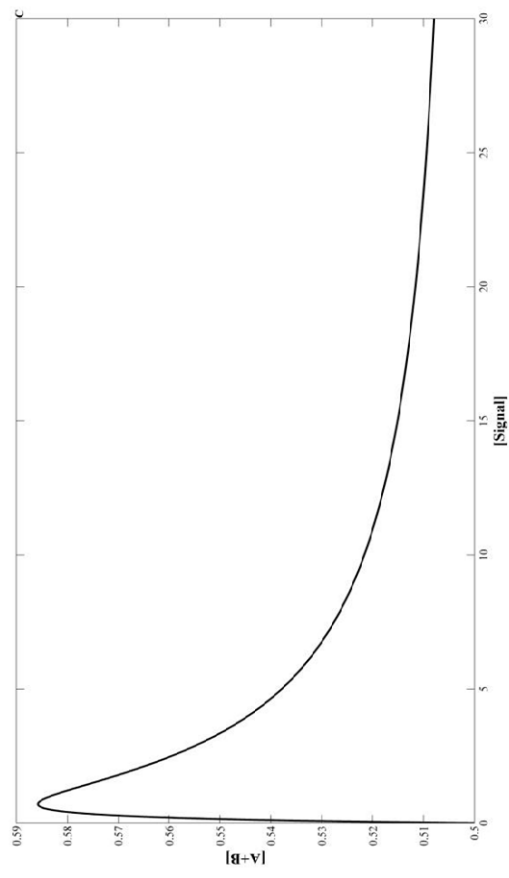
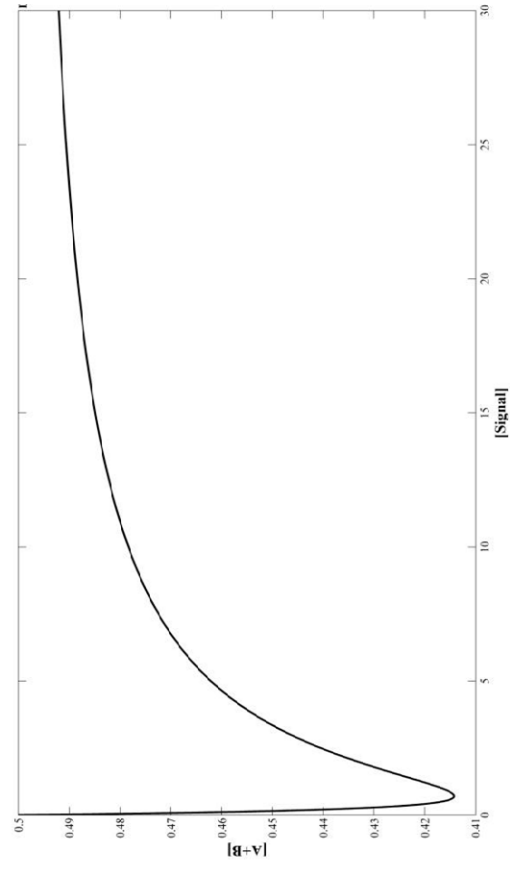
**C****D**

Fig.10

Figure 11

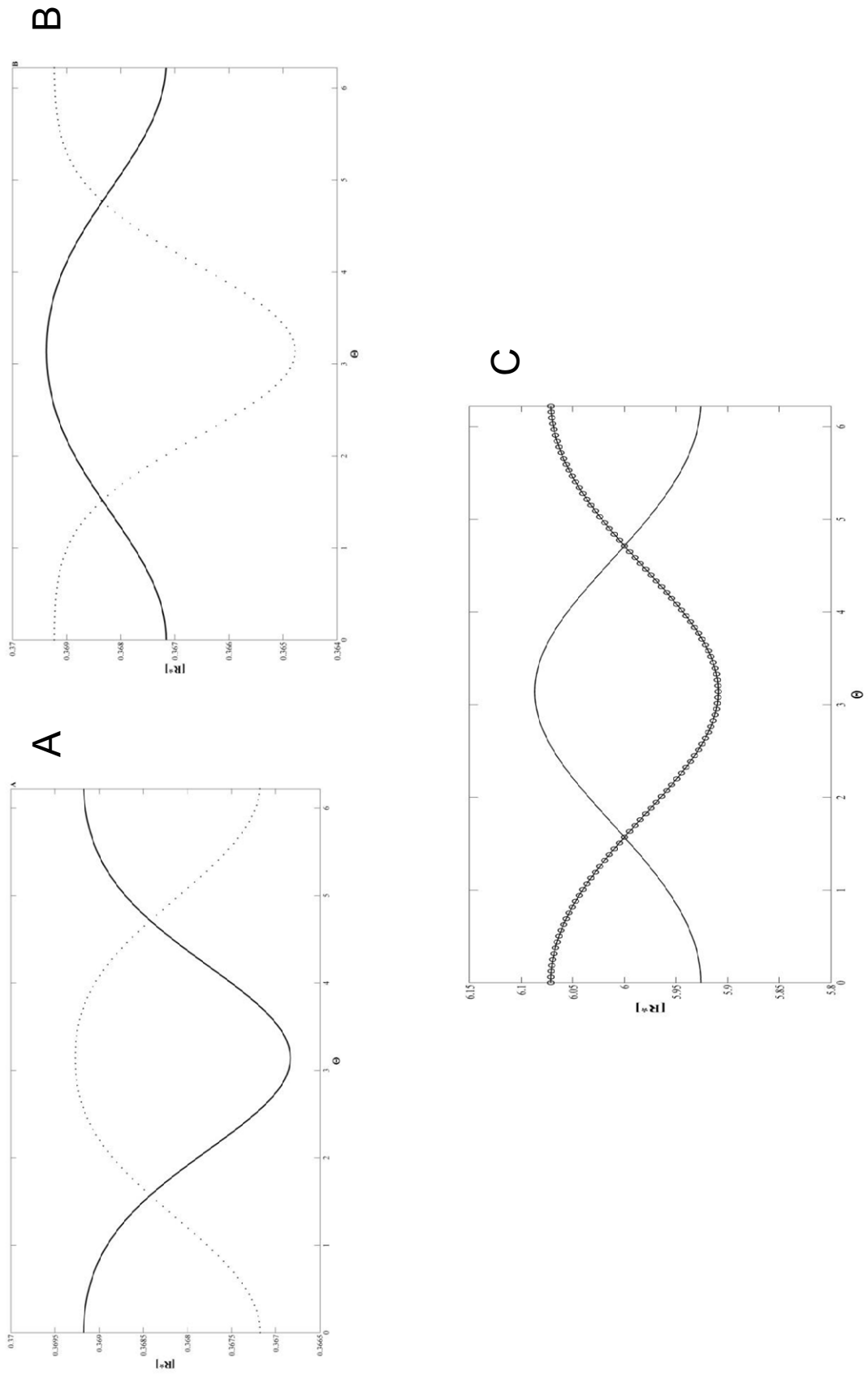


Fig.11

Figure 12

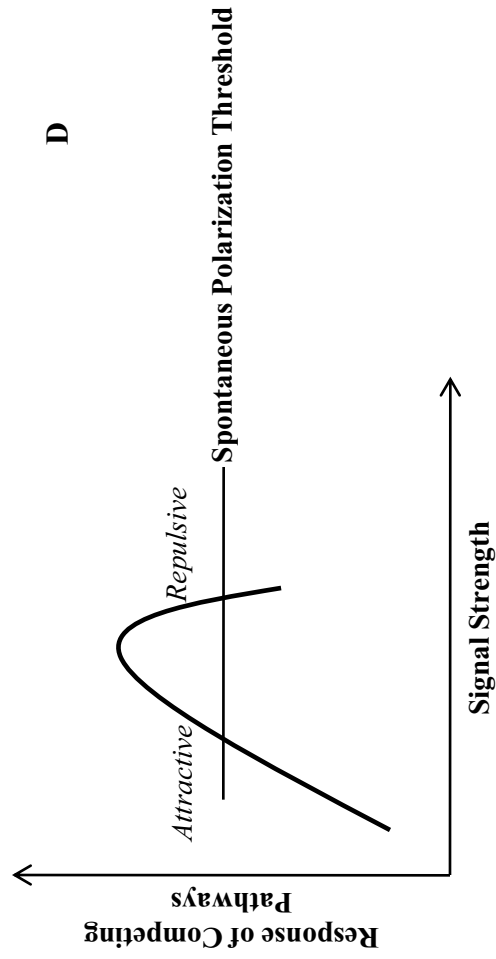
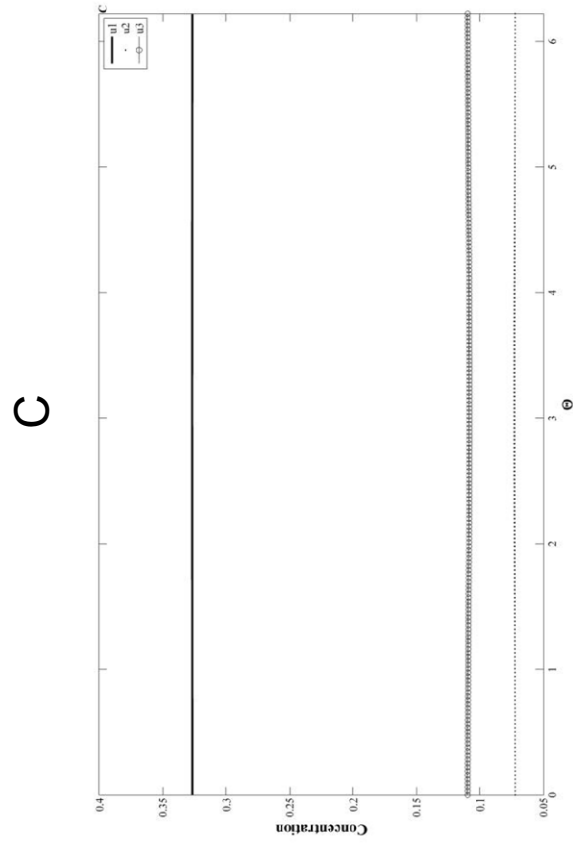
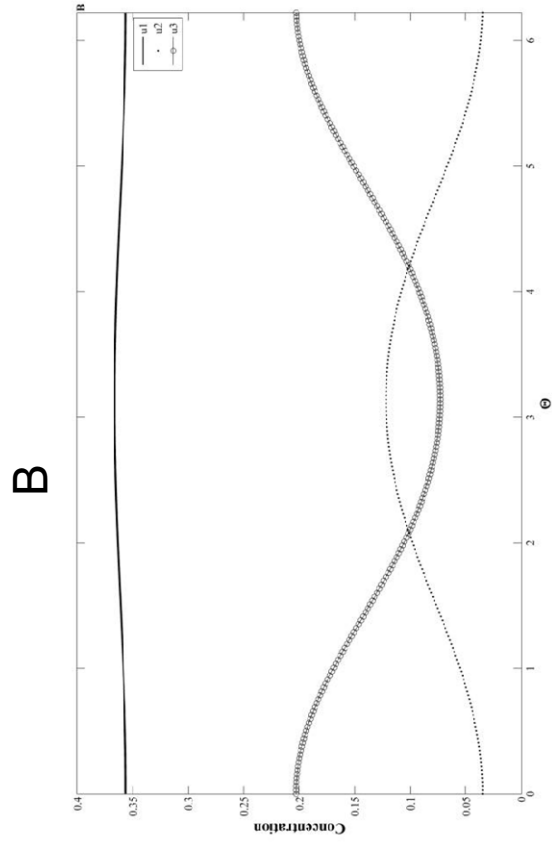
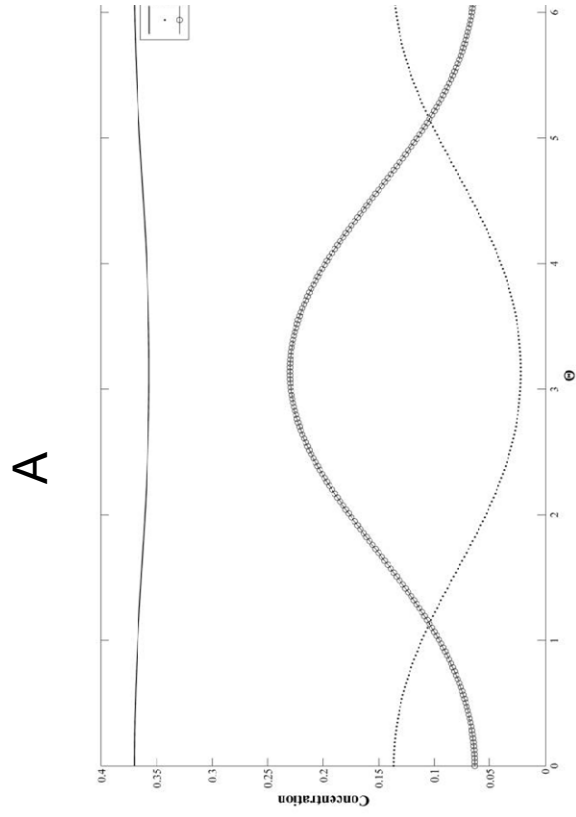


Fig.12

Design Feature		Downstream Signal Transduction Mechanism		
	Local Signalling	Adaptive Signalling	Spontaneous Polarization	
<p>Polarity Switch: Relevant to different CA & CR chemical</p>	<p><i>Switched biasing possible.</i> Effective switching for already polarized or moving cell.</p>	<p><i>Switched biasing.</i> Preserve/enhance switch effect by resetting mean value.</p>	<p><i>Switch in gradient response with qualitatively different features.</i></p> <p>Spontaneous polarization in homogeneous dose of CA but not CR (or vice versa). Homogenous CR dose can depolarize polarized cell.</p>	
<p>Competing Pathways: Relevant for the same chemical (CA & CR)</p>	<p><i>Switched gradient response as mean value of signal increased is possible.</i></p>	<p><i>Switched biasing</i> (same as previous)</p>	<p><i>Switched gradient response as mean value of signal is increased, combined with employment of non-employment of instability mechanism.</i></p> <p>Different combinations of attractive & repulsive gradient responses with & without instability mechanisms possible, as the mean value of the signal is increased.</p>	

Fig. 13

Figure 14

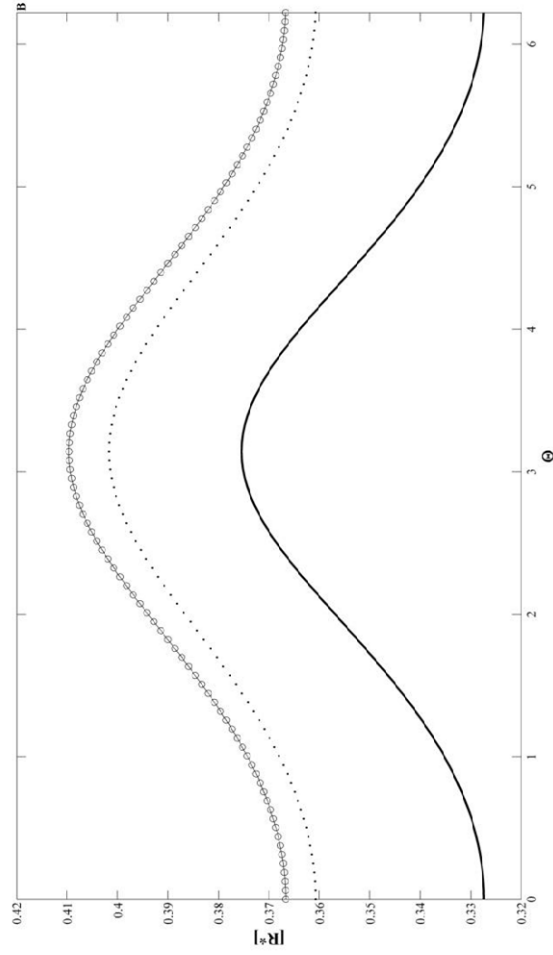
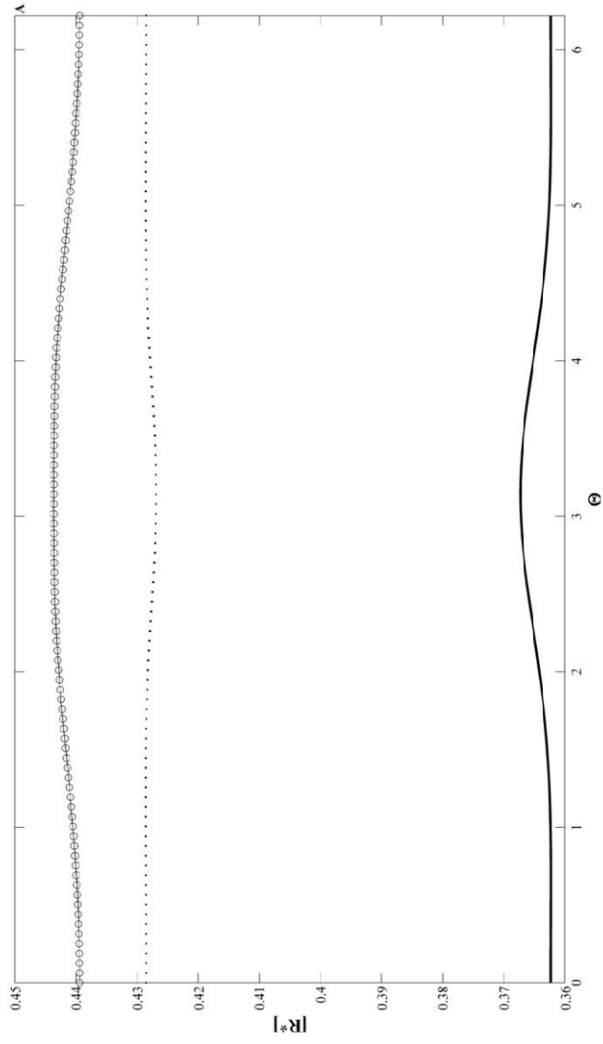


Fig. 14