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From Networks to Systems to Complex Systems: *A Signaling Pathway Coordination Case Study*

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Abstract—**Networks of signaling pathways provide a robust mechanism for cells to respond to various biological stimuli. In this paper we demonstrate cell adaptation through the viewpoint of an organizing principle between two interconnected pathways- MAPK and PKC. We use a multi-layered system representation of the pathways to determine the pathway components contributing to the adaptive behavior and coordination. The adaptation can be thought of as being manifested by a change in parameters of the coordinator. *In silico* experiments are conducted using MAPK/PKC mathematical model in literature to investigate the role of PLA_2 as a coordinator is reported here. Our results show that varying parameters of the coordinator not only activates the network of pathways where otherwise the pathway activity is very low, but also reveal the ability of the system to activate itself in the absence of the input, indicating relevance of the principle of bounded autonomy.**

I. Introduction

THE paradigm of a network is fundamental to understanding topology, connectivity, grouping, and clustering, among others, especially in biology [1]. The paradigm of a system can be thought of simply as 'a set of relations that evolve over time,' is but a starting point. Consequently, changes of rules of functioning in a system, is needed to address important biological phenomena such as adaptation, and resilience. Along with this, the paradigm of a complex system, viewed as a "system of systems," is needed to address the question of multilevel, hierarchical organization of biological phenomena and of the associated networks [2,3,6]. In this work, we demonstrate representation and analysis of networks into systems and subsequently complex systems for a network of Mitogen Activated Protein Kinase (MAPK) signaling pathways. We use a multi-layered system representation of the pathways to investigate which components of the system contribute to emergent properties such as adaptive behavior and coordination. In particular, we will examine a set of pathways consisting of Ras/Raf and Protein Kinase C (PKC) pathways and the interaction between them represented as cross-talk [3]. We explore the applicability of coordination principles and bounded autonomy to this instance [4]. The overall approach is to first establish the existence of a 'coordinator' in the system through a series of *in silico* experiments. This is reported in this paper. Forthcoming work will involve the validation of the coordinator through biological wet-lab experiments.

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II. Crosstalk Between Ras/raf and PKC pathways

Epidermal growth factor (EGF) and its receptor (EGFR–epidermal growth factor receptor), is one of the well-studied systems as it plays a vital role in producing various biological responses such as cell differentiation, division, motility, and growth, by specifying any of the numerous downstream pathways that it can activate. As the upstream regulator of the cellular pathways that promote cellular development, it is natural then that EGFR becomes the source target for cancer therapy [5].

MAPK and PKC pathways, which have been implicated in the cellular developmental processes, are such pathways that the EGF system activates among others. Transient activation of MAPK pathway through EGF triggers cell proliferation while persistent activation brings about differentiation [6]. Experiments have shown involvement of PKC in enhancing cell cycle progression and cell proliferation [7]. A pathway consists of molecular interactions that are not only restricted to activation of its downstream proteins, but also activation of proteins in another pathway, known as *cross-talk*, which can result in different biological responses from a single pathway activated alone. In this paper that we study cross-talk between EGF-activated MAPK and PKC pathway, where they form a positive feedback loop to regulate cellular development processes.

We chose to examine this functionally important pathway since mathematical models have been established and verified in literature by Bhalla and Iyengar through wet-lab

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experiments [8]. This we refer to as the Bhalla and Iyengar model henceforth.

II.1. Modeling MAPK and PKC Pathways

The model developed by Bhalla and Iyengar is illustrated in Figure 1. As it can be seen, interaction between MAPK and PKC is accommodated through Ras, Raf, and positive feedback from MAPK to PKC through PLA_2 and AA [8]. The illustrated model in Figure 1 is constructed using seven modules, which we will refer to as subsystems of biochemical reactions schemes (see [8] for more detailed reactions in each subsystem). There are two basic reaction scheme types involved in the subsystems: bimolecular reaction and Michaelis-Menten enzymatic reaction, where they are then formulated into nonlinear ODE (see Appendix for derivation). The resulting mathematical model consists of 127 state variables and more than one hundred parameters, which were obtained from published experimental data.

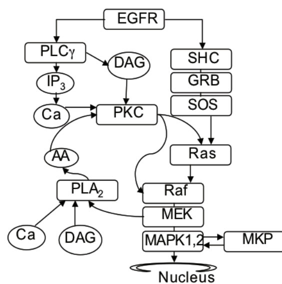


Figure 1. EGFR-activated MAPK and PKC pathway model developed by Bhalla and Iyengar [8]. Rectangles represent enzymes, and circle represents messenger molecules. The model is constructed using modules in [8] that illustrate biochemical interactions within the pathway (figure is reproduced from [8]).

II.2. Block Diagram of Pathways

A starting point for our analysis is the representation of the system using a block diagram as shown in Figure 2. Each block represents the subsystems that consist of the relevant biochemical reactions and the concentration pools of the corresponding species (denoted as state variables). The block diagram helps in identifying the causal flow of the signal that begins by a ligand (EGF) binding to the cell surface receptor (EGFR). In this model, EGFR is considered as a variable and therefore the number of available receptors is taken into consideration in the simulation.

As we can see clearly in Figure 2, the overall input to the system is EGF and the outputs are active MAPK (MAPK*) and active PKC (PKC*). We chose these two outputs because their signals are transmitted to the nucleus to initiate transcription activity of the targeted gene and eventually results in the desired cellular phenotype.

II.3. Hierarchical Representation of Pathways

To explore the existence of coordination in the MAPK-PKC pathway system described in Section II.1, we represent the block diagram of the system in Figure 1, as a hierarchical layers diagram (Figure 3). The system is divided into three hierarchical layers. The two interacting pathways are in Layer 2. They are coordinated by PLA_2 in Layer 1, while the interactions between the two pathways through Ras and Raf are in Layer 1. It is this interaction between PKC and MAPK (Layer 1) that receives extracellular input (EGF). PLA_2 is responsible to harmonize the processes on Layer 2, which is also acting as the delivery agent of the overall process to the nucleus.

Partitioning of layers in general is not unique and in our case it was chosen based on functional biological understanding and *in silico* experiments. We consider Layer 3, the PLA_2 subsystem, to be the coordinator of the pathway, a choice based on considerations of appropriateness to accept the coordination structure [2, 4]. From the viewpoint of Interaction Balance Principle (IBP) [4], MAPK* provides the necessary feedback information of the change in the interaction signals from Layer 1 to the third layer while AA provides the coordinating signal that will harmonize the interactions so that the overall goal of the system is achieved, making the PLA_2 subsystem a good candidate for a coordinator.

Accepting biochemical reactions as the structure of a model, the adaptation can be thought of as being manifested by a change in parameters. For example, if the environment influences the system in a persistent way, the coordinator will change a certain set of system parameters and consequently the output of a desired enzyme change in a commensurate perhaps non-linear, manner. In our analysis, we will explore how the parameter in the coordinator, PLA_2, changes as input is varied with the objective of the system responding in a bi-stable manner [8].

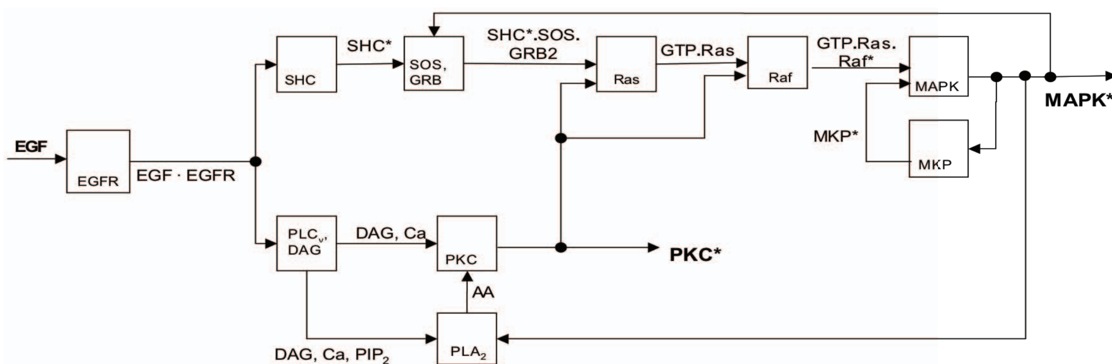


Figure 2. System's block diagram of PKC and MAPK pathways. System's input is EGF, and outputs are active MAPK (MAPK*) and active PKC (PKC*).

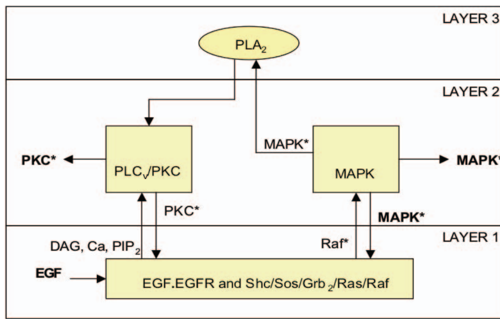


Figure 3. Hierarchical multi-layer diagram illustrating EGF activated MAPK/PKC pathway.

II.4. *Simulation Details and Validation*

The mathematical model is simulated using GENESIS/Kinetikit software (available in [9]) running on Sun Solaris. Euler exponential method [8] was used to solve the nonlinear ODE model numerically. We validated our simulation by reproducing Bhalla and Iyengar's simulation result that had been verified against wet-lab experimental data.

III. Exploration of the Coordination: Simulation Results

In order to explore PLA_2 as a coordinator of the system, various simulation experiments were conducted with a range of EGF input simulated as a rectangular pulse signal. The input is determined by (a) EGF concentration or the amplitude as measured in nM, and, (b) by the duration of the input as measured in minutes. Input is applied after letting the system relax for one hour.

The system output is measured separately through concentrations of active PKC (PKC*) and active MAPK (MAPK*). As it is shown by [8], and also confirmed by our *in silico* experiment results, the system is capable of exhibiting bistability behavior in its output. Bistability refers to the system's response where after input is withdrawn, the output does not reset to its pre-stimulation level, but rather retains its high concentration level. It is important to understand bistable behavior because transient activation and persistent activation (bistable response) of MAPK pathway brings about different cellular phenotype [6]. Bhalla and Iyengar [8] have also shown that bistability depends on both input amplitude and input duration, however they did not identify any threshold. Before we explore PKC as a coordinator, we conduct experiments to identify the bistability threshold for both input amplitude and duration, and analyze the relationship between these two input properties in the following section.

III.1. *Existence of Minimum Input Pulse Amplitude and Duration*

Our simulation experiment showed the existence of a minimum input pulse amplitude of 3.6 nM for which bistability occurs and carries along with it a minimum pulse duration. Figure 4A shows that applying an EGF input pulse amplitude of 3.5nM to the system, regardless of the input pulse duration, will not result in bistability. However, increasing input amplitude to 3.6 nM would result in bistable behavior (Figure 4B).

We can also see in Figure 4 that not only there exists an input amplitude threshold for bistability, but also input duration threshold exists, which is 47 minutes. When 3.6nM EGF is applied for 46 minutes or less, MAPK* and PKC* concentrations are reset to pre-stimulation levels upon recession of the input. Note that although 45 minutes is the half-life of EGF-EGFR complexes at least in one cell-line [13], wet-lab experiments indicated the possibility of stimulating the pathway with EGF for 100 minutes [8].

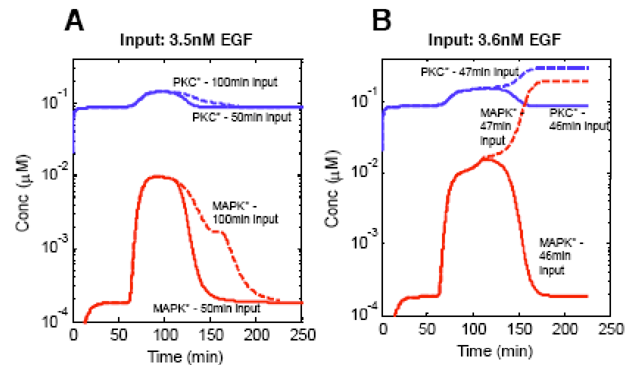


Figure 4. (A) No bistability occurs regardless input duration when input amplitude is set to 3.5 nM. (B) Input amplitude threshold for bistability is 3.6nM (with duration of 47 min).

The input duration threshold is not uniform across input amplitude. For a higher input amplitude value, input duration threshold decreases as shown for input amplitude of 5nM (Figure 5). For this particular stimulus, the input duration threshold was found to be 18 minutes, whereas for 3.6nM EGF the input duration threshold is 47 minutes.

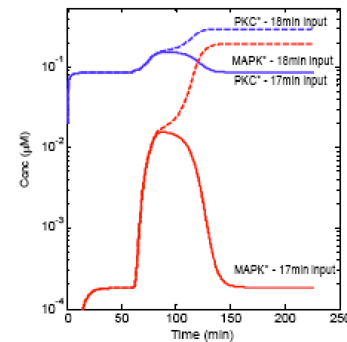


Figure 5. Input duration threshold for 5nM EGF. Bistability occurs when the stimulus is applied for 18 minutes or longer.

Dependence on both input pulse duration and amplitude can be thought about as "energy", simply the 'product or the integrand' of duration and amplitude. Our simulation revealed that the higher this 'energy', the faster the response. The following figure shows the systems response (MAPK* and PKC*) when input amplitude is set to 5 nM and the

input duration is varied from 10 min to 100 min. We can see that as input duration increases, MAPK* and PKC* reach high concentration value faster than that of lower input duration. Furthermore, at longer input duration, the response transiently peaked at higher concentration, while steady state leveled off to 0.2μM for MAPK* and 0.3μM for PKC*.

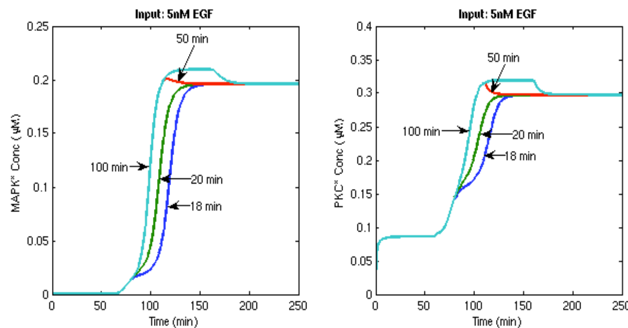


Figure 6. MAPK* (left) and PKC* (right) concentration when input is set to 5nM EGF with various duration.

III.2.PLA$_2$ Subsystem As A Coordinator

We focus our investigation now on exploring the possibility of PLA$_2$ subsystem acting as a coordinator. As shown in Figure 3, PLA2 subsystem is coordinating the overall system by balancing the interaction between PKC and MAPK subsystem. In order to better understand the role of PLA$_2$, we inhibited the interaction between PLA$_2$ and MAPK subsystems, and performed simulations under various inputs. Our results showed that the system did not exhibit any bistable responses regardless of high input duration and amplitude. Further changing the parameters in MAPK, PKC and PLC subsystems did not yield bistable responses. Therefore, we concluded that PLA$_2$ is indeed a coordinator for the overall system.

As a coordinator, PLA$_2$ adjusts the system's behavior so as to exhibit a bistable response under different input ranges. The adjustment is provided by varying parameters in PLA$_2$ subsystems from its nominal values. Here, nominal values refer to the parameter values in Bhalla and Iyengar model that were obtained from experimental result (available in [10]). In the subsequent simulations, we are searching for a parameter threshold, defined as the minimum parameter value that can bring about a bistable response across the input space. It is important to realize that we are varying the parameter values in ranges that are yet to be confirmed to be realistic using biological experiments.

As we mentioned earlier, two types of biochemical reactions in each subsystem are modeled: bimolecular and enzymatic reactions. At this time we will consider the enzymatic reactions, described by Michaelis-Menten constanst (K_m and V_{max}), in PLA$_2$ subsystem (See [11,12] for Michaelis-Menten constant derivation). In our simulation the Michaelis-Menten reaction constant is varied by changing V_{max} values in which will be referred as PLA$_2$ enzymatic constant henceforth (nominal V_{max} value is 120). The resulting PLA$_2$ enzymatic constant threshold is illustrated in Figure 7.

The shaded plane in Figure 7 demonstrates parameter threshold for a bistable response. For any input duration and amplitude, there exists a parameter value that can bring the system to a bistable behavior.

III.3.Bounded Autonomy

The parameter threshold illustrated in Figure 7 not only exists in the coordinating subsystem (PLA$_2$), but also in every subsystem. Using PKC as an example, we will first observe the behavior of the system when the constant for bimolecular reactions are varied, and then we will explore the enzymatic reaction constant to show the existence of such a threshold.

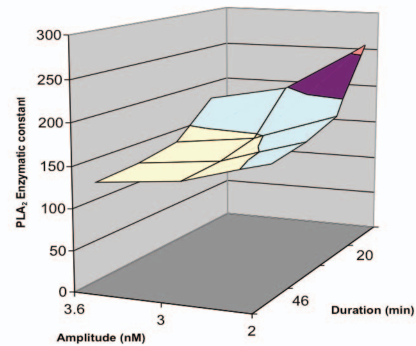


Figure 7. PLA$_2$ enzymatic constant – bistable response threshold. Setting enzymatic constant above the area will result in bistable behavior, while parameters below the area will result in transient activation only. Nominal value of the enzymatic constant is 120.

Bimolecular reaction uses two constants, forward reaction constant (k_f) and backward reaction constant (k_b). In the following simulation, we chose to vary the smallest backward and forward constant ratio in PKC subsystem since it will be more sensitive to the changes. The nominal value of the selected forward reaction constant is 1. The goal in this simulation is to find forward reaction constant that is capable of bringing bistability response for an input in which at nominal value will not yield bistability. We selected such input to be 3.6 nM for 40 minutes. Based on our simulation, raising the forward reaction constant to 3 results in bistability behavior, and more interestingly, setting k_f to an even higher value of 10, gave a bistability response which occurred before stimulus was applied, and hence "Auto-Activation" (Figure 8). Furthermore, the steady state concentration value of PKC* and MAPK* is higher when k_f is set to 10.

Now we will turn to the enzymatic reaction constant. In the subsequent simulations, we varied Michaelis-Menten enzymatic parameters in a reaction that involves PKC. This reaction exists in MAPK subsystem and Ras subsystem, where PKC acts as an enzyme for Raf and Ras respectively. Again, in our simulation the Michaelis-Menten reaction constant is varied by changing V_{max} values which will be referred to PKC enzymatic constant henceforth. Nominal values of PKC enzymatic constant in both subsystems are 4.

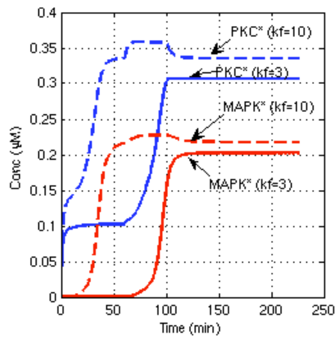


Figure 8. Input is 3.6nM EGF for 40 minutes, applied when time is 60 minutes. Varying forward reaction constant, k_f, in the bimolecular reaction of PKC subsystem (nominal value is 1) to 3 results in bistability, while raising the constant to 10 gave auto-activation.

Let us now consider the PKC enzymatic reaction constant in MAPK subsystem (PKC-Raf reaction). We simulated the system with EGF input of 5nM for 10 minutes, where at nominal PKC enzymatic constant value the system does not exhibit bistability behavior. Our simulation revealed that for such input amplitude and duration, the minimum PKC enzymatic constant in MAPK subsystem required for bistability is 5. Setting PKC enzymatic reaction constant in Ras subsystem to 5, on the other hand, did not yield any bistability behavior. The PKC enzymatic constant in Ras subsystem needs to be fixed at higher value, at least 6, for bistability to occur. These results are shown in Table 1.

Similar to bimolecular reaction constants, changing PKC enzymatic constant does not only result in bistability but also "auto-activation". Through our simulation we identify the minimum required PKC enzymatic constant in both MAPK and Ras subsystems for "auto-activation". Our results show that the required minimum PKC enzymatic constant value for MAPK subsystem is 8, while that of Ras subsystem is 13. This means that there is enough 'amplification' due to a positive feedback mechanism for the system to automatically switch to a bi-stable response state.

Table 1. Bistability threshold for PKC enzymatic constant value. Nominal value for the constant in both subsystems is 4.

INPUT	MAPK-subsystem	Ras subsystem
5 nM for 10 min	5	nominal value
	nominal value	6
no input	8	nominal value
	nominal value	13
	6	6

Subsequently, we varied PKC enzymatic constant in both subsystems simultaneously to search for the minimum constant value for auto-activation. Our simulation showed that when the constant in both subsystems is altered, the auto-activation threshold is lowered. In both subsystems, PKC enzymatic constant should be set to at least 6, as shown in Table 1.

Continuing the simulations in search of minimum parameter values in MAPK subsystem that preserve bistable response results in threshold plane illustrated in Figure 9. As we can see, there exists a range of key parameters where categorical bistability behavior is preserved for any given input (amplitude and duration), and the domain of this parameter is the 'bounded autonomy' domain, which provides MAPK subsystem adaptation ability in terms of endogeneous changes in the parameter. As long as the parameters are within this bounded autonomy domain, the coordinator will not interfere on the lower level subsystem (MAPK and PKC for example).

If we take a slice across input duration (of Figure 9), we can see that the more active adaptation is needed for short duration view where endogenous change of PKC enzymatic parameter is needed (Figure 10A). Steepness of adaptation for short duration is shown in Figure 10B. For input over longer time period, adaptation is hardly necessary.

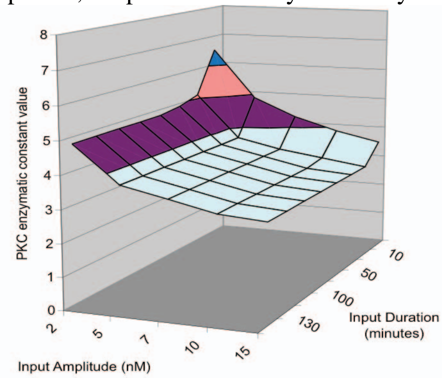


Figure 9. Bistability threshold of PKC enzymatic constant for MAPK subsystem.

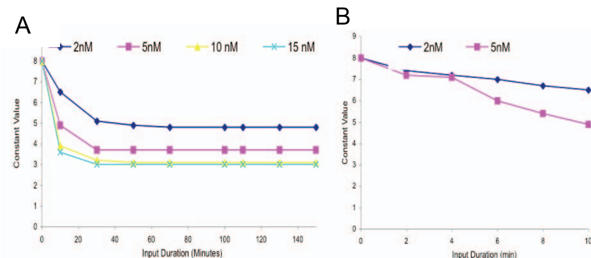


Figure 10. (A) PKC enzymatic constant bistability threshold for various input amplitude and duration. Slope of the shorter time duration (B).

Similar behavior is found in reference to changes in the input amplitude (Figure 11). Here, adaptation required over a broader range of amplitude values comparing to the ranges for use of time duration changes (Figure 10).

Based on these parametric simulations, we can see that low input duration and low input amplitude requires more adjustment in the PKC enzymatic constant so as to exhibit a bistable response. At higher input amplitude and duration, PKC enzymatic constant does not need to be adjusted since the input "energy" is enough to activate the positive feedback loop and thereby exhibiting bistability. An interesting observation from Figure 8 and 9 is that in the absence of input (EGF is set to 0 nM), bistability will occur if the parameter is increased to 8. Increasing the parameter to such a high value may not be physically feasible, and therefore further investigation on refining the model is required.

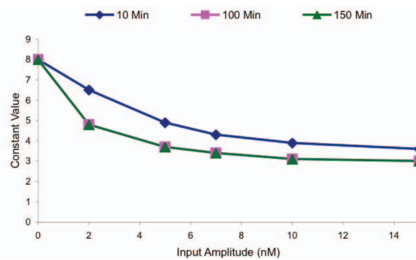


Figure 11. PKC enzymatic constant threshold in MAPK subsystem for range of input amplitude.

IV. CONCLUSION

Throughout this paper we have presented our analysis of a signaling pathway, specifically EGF-activated MAPK/PKC pathway, through regulation, adaptation, and coordination point of view. We represent the pathway using a model developed by Bhalla and Iyengar [8] as a hierarchical multi-layered diagram (Figure 3) enabling us to explore the existence of coordination within the pathway. On the hierarchical layers diagram, PLA_2 is in a position to coordinate or harmonize PKC and MAPK subsystems. In turn, MAPK and PKC are capable of performing adaptive function in response to the changes in input (EGF). We then further suggest that PLA_2 acts as the coordinator which is responsible for adjusting the overall systems behavior, manifested by endogenous change in PLA_2 parameters, in response to various inputs. Theoretical proof of this will be work in the sequel.

Our first analysis is to find the minimum input amplitude and duration for bistability, as Bhalla and Iyengar [8] has shown in their work that bistability threshold does not only depend on input duration but also input amplitude. We found that the minimum amplitude for bistability is 3.6nM EGF, and at this amplitude level, the input duration threshold is 47 minutes. However, input duration threshold is not universal; as amplitude increases, input duration threshold decreases. Combining these two input features, amplitude and duration, as ‘energy’, shows that the higher this ‘energy’, the faster the system reaches its peak and thereby exhibiting bistability response at shorter time. Whether such threshold is compatible with biological reality needs to be further verified through wet-lab experiments

Through our *in silico* experiments we explore the system behavior under various parameter values and for a range of input. We identify the minimum required PKC enzymatic and bimolecular forward constant for bistability and auto-activation. The minimum value for bistability of PKC bimolecular forward reaction constant with 10 minutes of 3.6nM EGF is 3, while the minimum value for auto-activation is 10. Our analysis showed that PKC enzymatic constant in MAPK subsystem is more sensitive than in Ras subsystem, since the minimum PKC enzymatic constant value for bistability is lower in MAPK subsystem.

Further analysis on the PKC enzymatic constant demonstrated the existence of ‘bounded autonomy’, providing a range for key parameter that will preserve the categorical bistability response for any given input. Varying parameters in the pathways can also be considered to represent the effect of discounted dynamics in the model. This points out a need to conduct control *in vivo* experiments to ascertain whether the pathways can change in reality as the *in silico* experiments indicate.

APPENDIX

Bimolecular Reaction

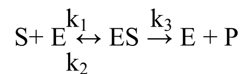
A+B↔AB, with forward reaction k_f and backward reaction k_b

Nonlinear ODE formulation:

$$\frac{d(A)}{dt} = k_d\,(AB) - k_f\,(A)(B) \qquad (1)$$

$$\frac{d(AB)}{dt} = k_f\,(A)(B) = k_d\,(AB) \qquad (2)$$

Michaelis-Menten Enzymatic Reaction



$$S + E \underset{k_2}{\overset{k_1}{\leftrightarrow}} ES \overset{k_3}{\rightarrow} E + P$$

ODE formulation is the same as bimolecular reaction above.

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