Cell signaling final report
Computational modelling of the MAPK signaling cascade

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I. INTRODUCTION

In this report, a study of the MAPK cascade is carried out through a 4th order Runge-Kutta algorithm. The MAPK cascade is a complex signaling pathway of interacting proteins and enzymes that regulate cell behaviour, and working with computational algorithms helps understand the dynamics of this signalling process, and predicting its behaviour.

Our model simulates the time course of the concentrations of the signaling molecules and the phosphorylation status of the various enzymes in the pathway under different conditions, including varying concentrations of the activators. The results provide insights into the complex dynamics of the MAPK cascade and its response to different stimuli.

The code has been written in $fortran90$ and the plots have been made with *Python*.

II. INTRODUCTION TO SIGNALING PROCESSES

Cell signaling is the process by which cells communicate with one another within the body by releasing and receiving hormones and other signaling molecules. Cell signaling can occur via a variety of pathways, but the common element is that the actions of one cell influence the function of another. Multicellular organisms rely on cell signaling to coordinate a wide variety of functions.

Certain types of cell signaling occur intracellularly, whereas others occur intercellularly. The same cell that receives the signal generates intracellular signals. Intercellular signals, on the other hand, can travel throughout the body. This enables particular glands within the body to produce signals that act on a variety of tissues across the body. The required receptors will be present on each target cell.

This way, cell signalling can be separated into three different stages. Initially, there is reception, in which the signal molecule attaches to the receptor. Then comes the signal transduction, in which the chemical signal causes a cascade of enzyme activations. Finally, there is the response, which is the outcome of the cellular responses.

In each step of a signalling cascade, there is an activation or deactivation of an intracellular signaling protein, where an enzymatic-like reaction occurs. The activated protein works as the catalyst that activates the following signalling protein, and so on. The deactivation process works the same way, but backwards.

III. THE MAPK SIGNALING CASCADE

MAPK cascades are essential signaling pathways that regulate a wide range of activated cellular activities such as proliferation, differentiation, apoptosis, and stress response. As a result, dysregulation, or incorrect functioning of these cascades, plays a part in the development and progression of diseases including as cancer, diabetes, autoimmune diseases, and developmental abnormalities.

Mitogen activated protein kinases (MAPK) are a family of kinases that respond to a variety of extracellular signals by chemically adding a phosphate group though a process of phosphorylation, and act as an on/off switch. Kinases are a family of enzymes that catalyze the process of phosphorylation of proteins by consuming ATP or GTP (organic compounds that provide energy to chemical reactions).

The MAPK cascade typically begins with the activation of a cell surface receptor by an extracellular stimulus. This leads to the activation of a protein kinase, called a MAP kinase kinase kinase (MAPKKK), which in turn activates a MAP kinase kinase (MAPKK) by double phosphorylation (PP). The MAPKK then activates a MAP kinase (MAPK) by double phosphorylation again. Finally, the MAPK can phosphorylate and activate target regulatory proteins, including transcription factors, leading to changes in gene expression. The general scheme of this cascade is shown in figure 1.

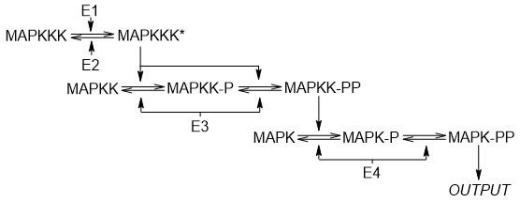


Figure 1: Schematic representation of the MAPK cascade. The four enzymes that participate in the

reaction are labeled as E1, E2, E3 and E4. The number of phoshorylations of the proteins is inidicated by the number of P or by the number of $*$.

IV. EFFECTS OF THE CASCADE SEQUENCE ON ROBUSTNESS AND SENSITIVITY OF SIGNAL TRANSDUCTION

The MAPK cascade can play a role in regulating the robustness and sensitivity of signal transduction in cells.

The ability of a signaling pathway to work reliably under different conditions, such as changes in signal concentration or duration, is referred to as robustness. The MAPK cascade can help with robustness by acting as a signal amplification mechanism. This amplification can take place through several steps of kinase activation, resulting in a greater signal output than the initial input. Moreover, the MAPK cascade can trigger negative feedback loops, which can help to moderate the signal and prevent overactivation.

Sensitivity refers to the ability of a signaling pathway to detect and respond to changes in the concentration or duration of a signal. The MAPK cascade can contribute to sensitivity by providing a mechanism for signal integration. For example, if multiple extracellular signals are received simultaneously, they can be integrated by the MAPK cascade, leading to a coordinated cellular response.

In conclusion, the MAPK cascade can improve the robustness and sensitivity of signal transduction in cells via mechanisms such as signal amplification, negative feedback, and signal integration. These MAPK cascade features enable cells to respond effectively to external stimuli under varied conditions.

A. The model and equations

The first step of the MAPK cascade is the phosphorylation of MAPKKK. The MAPKKK binds with the first enzyme (E1) forming a complex (MAPKKK-E1), having a rate constant a1 (association). The process is reversible, and the backwards reaction has a rate constant d1 (dissociation). Once the complex is created, it reacts and the resulting products are an activated MAPKKK with a phosphate (MAPKKK-P) and the enzyme E1, having a rate constant k1 (product formation). This whole process is represented in the first step of figure 2.

The reverse process is also possible, and it is represented in the second step of figure 2. The phosphorylated MAPKKK binds with a second enzyme (E2) and forms a complex (MAPKKK-E1) with a rate constant a2 and d2 for the reverse reaction. This complex then dissociates into the same enzyme E2 and the original protein MAPKKK with a rate constant k2.

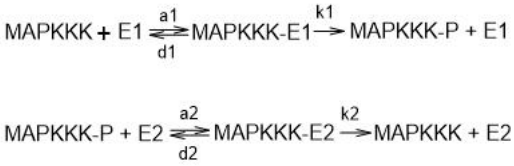


Figure 2: First step of the MAPK cascade reactions.

The following steps of the cascade follow the same scheme as this first initial process, having a second step where the MAPKK is activated by double phosphorylation and through an enzyme E3, and finally a third step where the MAPK is also activated by double phosphorylation and through an enzyme E4. Altogether, there are a total of 10 reactions.

These reactions described above give rise to 18 rate equations for each compound that participates in the cascade. Equations (1), (2), (3) and (4), represent the first two reactions of figure 2, without taking into account the subsequent reactions of the cascade.

$$\frac{d}{dt}[KKK] = -a_1[KKK][E1] + d_1[KKK \cdot E1] + k_2[KKK^* \cdot E2] \quad (1)$$

$$\frac{d}{dt}[KKK \cdot E1] = a_1[KKK][E1] - (d_1 + k_1)[EEE \cdot E1] \quad (2)$$

$$\frac{d}{dt}[KKK^*] = -a_2[KKK^*][E2] + d_2[KKK^* \cdot E2] + k1[KKK \cdot E1] \quad (3)$$

$$\frac{d}{dt}[KKK^* \cdot E2] = a_2[KKK^*][E2] - (d_2 + k_2)[KKK^* \cdot E2] \quad (4)$$

There are also mass conservation equations for the four different enzymes that participate in the cascade:

$$[E1] = [E1_{tot}] - [KKK \cdot E1] \quad (5)$$

$$[E2] = [E2_{tot}] - [KKK^* \cdot E2] \quad (6)$$

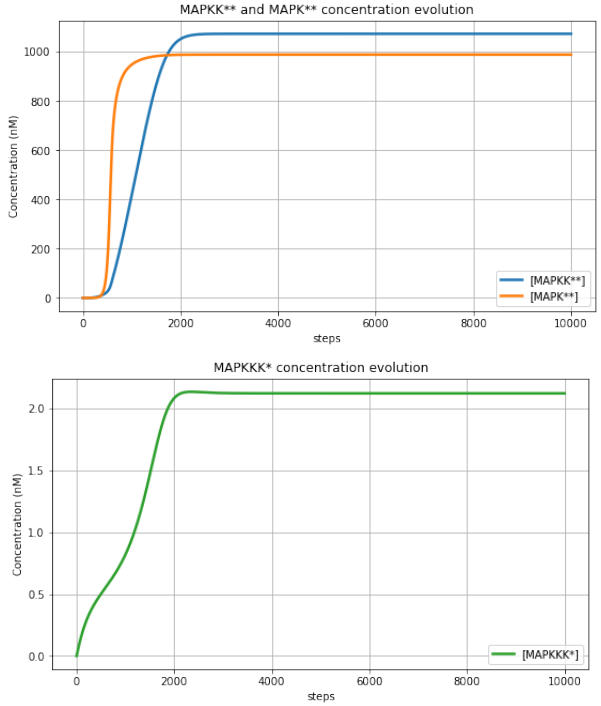
$$[E3] = [KKP'ase] = [KKP'ase_{tot}] - [KKP'ase \cdot KK - P] - [KKP'ase \cdot KK - PP] \quad (7)$$

$$[E4] = [KP'ase] = [KP'ase_{tot}] - [K - P \cdot KP'ase] - [K - PP \cdot KP'ase] \quad (8)$$

B. Results

A 4^{th} order Runge-Kutta Algorithm is used to simulate the MAPK cascade. First of all, a numerical resolution is need to solve the 18 differential equations as well as the four mass conservation equations. The following initial values have been used: $[E1]_{t=0} = 1$, $[E2]_{t=0} = [E3]_{t=0} = 0.3$, $[E4]_{t=0} = 120$, $[MAPKKK]_{t=0} = 3$, $[MAPKK]_{t=0} = [MAPK]_{t=0} = 1200$, $a_i = 1/150$ and $d_i = k_i = 1$. All the concentrations are in nanomolar (nM) units.

The temporal evolution of MAPKKK*, MAPKK* and MAPK* is then plotted, for a final time $t_f = 10,000$ steps and in steps of 0.01 units. The plots are shown in figures 3 and 4.



Figures 3 and 4: Temporal evolution of MAPKKK*, MAPKK** and MAPK**

It is clear how the three different activated compounds reach a steady state after around 2000 steps. The stationary values for each compound are: 2.138 nM for MAPKKK*, 1073.629 nM for MAPKK** and 988.912 nM for MAPK**.

Now that the stationary values for each compound have been computed, we can recalculate these values for an initial stimulus of E1 ranging from 0 to 1 (instead of a fixed values of 1 like before), and again in steps of 0.01 units. The following stationary values are obtained: 2.758 nM for MAPKKK*, 1081.255 nM for MAPKK** and 989.094 nM for MAPK*.

Finally the stationary values of the three activated kinases vs $[E1]_{t=0}$ are plotted, and we re-scale them so that the input stimulus is expressed in multiples of the EC_{50}, which is the concentration of $E1_{tot}$ that produces a 50% maximal response. The plot is shown in figure 5.

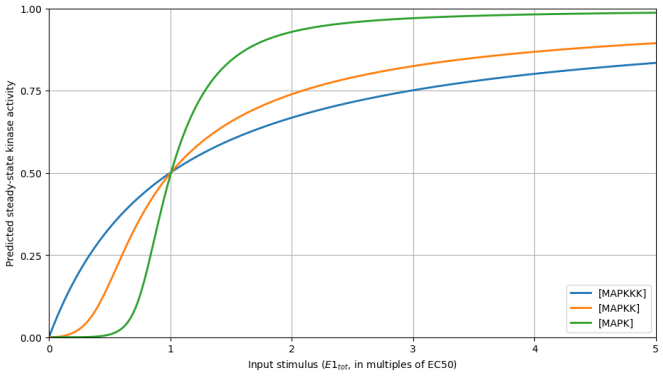


Figure 5: Predicted response curves for MAPK cascade, where the input stimulus is expressed in multiples of the EC_{50}

C. Discussion

As shown in figure 5, the stimulus/response curve for MAPKKK* is hyperbolic, while the curves for MAPKK** and MAPK** are sigmoidal.

The hyperbolic curve of MAPKKK* implies that its concentration reaches a maximum value at a particular concentration of the input stimulus E1, and beyond that concentration, the concentration of MAPKKK* remains constant. This behavior is typical of enzymes that follow Michaelis-Menten kinetics, where the rate of the reaction reaches a maximum at a particular substrate concentration, and beyond that concentration, the rate of the reaction remains constant.

The sigmoidal curves of MAPKK** and MAPK** suggest that their concentration increases gradually at lower concentrations of the input stimulus E1, reaches a maximum value at an intermediate concentration, and then saturates at higher concentrations. This behavior means that the MAPKK** and MAPK** may be regulated by the binding of another molecule that modifies their activity. This promotes ultra-sensitivity of the stimulus/response behaviour.

Overall, the different shapes of the concentration curves for MAPKKK*, MAPKK**, and MAPK** suggest that these proteins have distinct regulatory mechanisms that determine their response to the input stimulus E1.

V. CONCLUSIONS

Through our analysis, we have gained insights into the complex dynamics of the MAPK cascade and its response to different stimuli. We have demonstrated the utility of the 4th order Runge-Kutta method in accurately simulating the behavior of biochemical reactions and shown that our model provides a useful tool for predicting the effects of perturbations on the MAPK cascade. The results obtained in this report match with those displayed in the article by Huang CY and Ferrell JE Jr. "Ultrasensivity in the mitogen-activated protein kinase cascade" [1], and therefore we can conclude that our results are correct.

[1] Huang CY, Ferrell JE Jr. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc Natl Acad Sci U S A. 1996 Sep 17;93(19):10078-83. doi: 10.1073/pnas.93.19.10078. PMID: 8816754; PMCID: PMC38339.