MODELING CELLULAR SIGNAL PROCESSING USING INTERACTING MARKOV CHAINS

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ABSTRACT

Signal processing is an integral part of cell biology. The associated algorithms are implemented by signaling pathways that cell biologists are just beginning to understand and characterize. Our objective in the context of signal processing is to understand these algorithms and perhaps emulate them in other contexts such as communication and speech processing. Towards this end, this paper proposes a new framework for modeling cellular signal processing using interacting Markov chains. The model is presented and preliminary results that validate it are given. Specifically, the example of the mitogen activated protein kinase cascade is examined and model predictions are compared to experimental findings. The model is consistent with the key properties of the cascade, i.e. ultrasensitivity, adaptation, and bistability.

1. INTRODUCTION

Biology presents a potentially very fruitful metaphor for signal processing. In fact, there is very strong evidence of interesting sophisticated signal processing operations performed by living systems including ones reminiscent of frequency modulation coding [1] and multi-resolution filterbanks. Biological signaling, in particular, takes on different forms ranging from electrical signals through nerve synapses, physical signals such as mechanical stress or pressure at the surface of cells, to chemical signals such as hormone concentrations in the bloodstream. While some of these signals, notably electrical and physical signals, have been historically easier to study and control than others, the emergence of high throughput technologies for molecular biology is making the study of biochemical signaling networks a possibility. In addition, as more signaling networks and elements are identified, their complexities and the intricate interactions among signaling molecules, or cross-talk, are quickly becoming intractable. Understanding how cells do signal processing therefore eventually requires models that define layers of abstractions in order to view signaling algorithms at different resolutions.

Most current models for signaling pathways can be classified in one of two categories: biochemical models which define the system in terms of kinetic differential equations governed by the laws of mass action or Michaelis Menten kinetics and biophysical models which are concerned with the mechanical forces and spatial distributions necessary to generate a response. While these models usually have a direct physical basis, even the simplest of these includes hundreds of equations and many parameters that usually need to be estimated since they are not directly measurable. As a result, one can rarely gain intuition from such models. Also, the under-constrained parameter space makes such models somewhat arbitrary.

In this paper, we propose a fundamentally different approach to modeling based on statistical control and signal processing theory. Our goal is to develop an intuition as well as some understanding of how cells perform signal processing based on *approximate* models inspired from our engineered signal processing networks. We limit our approach to signaling networks within cells. The intracellular signaling networks we are interested in are composed of proteins and enzymes as well as other molecules such as DNA, phosphates, and ATP. Signaling usually occurs through a series of protein modifications such as phosphorylation (the addition of phosphate groups) and cleavage, translocation from the cytoplasm to the nucleus, as well as control of gene expression. Usually, signals propagate through cascades where one protein affects the activity of another downstream protein. However, cross-talk also plays an important role in the signaling mechanism where several proteins coming from different upstream signals affect the same downstream signal or a set of different downstream signals.

In the following sections, we first present a model of interacting Markov chains in Section 2, we then give, in Section 3, an example of an evolutionary conserved biological signaling network and discuss its properties. Finally, in Section 4, we apply our modeling approach to this network and present model predictions and results.

2. INTERACTING MARKOV CHAINS

2.1. Model Formulation

Our model is composed of a network of v interacting nodes. Each node is composed of a k-state non-homogeneous Markov chain, i.e. the state $X_p[n]$ of node X_p at time n is given by:

$$P(X_p[n]=j|X_p[n-1]=i,\cdots,X_p[0]=m)=P(X_p[n]=j|X_p[n-1]=i)=p_{ij}^{X_p}[n]$$

In the absence of any interactions, the transition probabilities of chain X_p are simply $p_{ij}^{X_p}[n]=q_{ij}^{X_p}[n]$ where $q_{ij}^{X_p}[n]$ is independent of all states in the network. More generally, interactions between different nodes are defined by influences of states in one node onto transition probabilities in another node. This influence may be either positive (activating) or negative (inhibiting). Specifically, if a given state l in node X_r influences the transition probability from state i to state j in node X_p then the transition probability from state i to state j in node X_p at time n due to the influence of state l in X_r, $\{p_{ij}^{X_p}[n]\}_{[l,X_r]}$ is given by:

$$\{p_{ij}^{X_p}[n]\}_{[l,X_r]}=\alpha_{r,l}f\{P(X_r[n-1]=l)\}^{\beta_{r,l}} \quad (1)$$

where $P(X_r[n-1]=l)$ is the *a-priori* probability of node X_r being in state l at time $n-1$, $0\leq\alpha_{r,l}\leq 1$ and $0<\beta_{r,l}$ are constants, and $f\{x\}=x$ if the influence is positive, otherwise $f\{x\}=1-x$ if the influence is negative. Furthermore, in addition to $q_{ij}^{X_p}[n]$, a given transition probability may be subject to influences from several states in different nodes, each one of which will have a form similar to equa-

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tion (1). There are clearly many different ways in which the individual transition probabilities, or equivalently the influences, may be combined with $q_{ij}^{X_p}[n]$ into one composite transition probability. Here we consider two models for combining the state influences: a weighted average model and a fading model.

2.1.1. Weighted average model

As the name suggests, in the weighted average model, the influence of states from different nodes are added together to form $p_{ij}^{X_p}[n]$, the total transition probability from state i to state j in node X_p at time n as follows:

$$p_{ij}^{X_p}[n]=\sum_r \sum_l \frac{\{p_{ij}^{X_p}[n]\}_{[l,X_r]}+\alpha_{q,p}(q_{ij}^{X_p}[n])^{\beta_{q,p}}}{\sum_r \sum_l \alpha_{r,l}+\alpha_{q,p}} \quad (2)$$

where $0\leq\alpha_{q,p}\leq 1$ and $0\leq\beta_{q,p}$ are constants.

2.1.2. Fading model

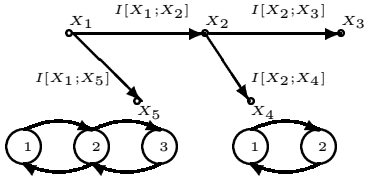
In the fading model, the individual influences are combined through multiplication, i.e. the composite probability is given by:

$$p_{ij}^{X_p}[n]=\prod_{r,l}\{p_{ij}^{X_p}[n]\}_{[l,X_r]}\alpha_{q,p}(q_{ij}^{X_p}[n])^{\beta_{q,p}} \quad (3)$$

where again $0\leq\alpha_{q,p}\leq 1$, and $0\leq\beta_{q,p}$.

2.1.3. Notation

A graphic representation of the model is given in Figure 1 where directed arrows between nodes define influences of specific states in the originating node onto specific transition probabilities in the receiving node. The influence is labeled $I[X_r,X_p]$ where X_r is the originating node and X_p is the receiving node. Each $I[X_r,X_p]$ carries with it a set of relations of the form $[l,X_r]\xrightarrow{\gamma}[p_{ij},X_p]$ where l is the state in node X_r influencing transition probability p_{ij} in node X_p and $\gamma=+$ if the influence is positive, otherwise $\gamma=-$ if the influence is negative. Examples of such influences are given in Figure 1.



$I[X_1,X_2] = [2,X_1]\xrightarrow{+}[p_{12},X_2]$
$I[X_2,X_3] = [1,X_2]\xrightarrow{+}[p_{21},X_3]$
$I[X_1,X_5] = [2,X_1]\xrightarrow{-}[p_{23},X_5]$
$I[X_2,X_4] = [1,X_2]\xrightarrow{-}[p_{11},X_4]$
$[3,X_2]\xrightarrow{+}[p_{21},X_4]$

Fig. 1. Graphical representation of the interacting Markov chain model. Two representative Markov chains are illustrated for nodes 4 and 5: a two-state and a three-state chain respectively.

2.1.4. Evolution of the state probabilities

Recursive formulas for the state occupancy probabilities of the individual Markov chains are similar to those of traditional Markov chains [2]. Specifically, let $\mathbf{p}_{X_r}[n]$ be a row vector of length k where k is the order of the Markov chain at node X_r and whose entries correspond to the state probabilities at time n, i.e. the i-th entry corresponds to the *a priori* probability of chain X_r being in state l at time n, $P(X_r[n]=l)$, and the initial state probability distribution is given by $\mathbf{p}_{X_r}[0]$. Furthermore, let $\mathbf{A}_{X_rX_r}[n]$ be the transition matrix of the Markov chain at node X_r at time n, i.e. $\mathbf{A}_{X_rX_r}[n]$ is written as follows:

$$\mathbf{A}_{X_rX_r}[n]=\begin{bmatrix} p_{11}^{X_r}[n] & \cdots & p_{1k}^{X_r}[n] \\ \vdots & \ddots & \vdots \\ p_{k1}^{X_r}[n] & \cdots & p_{kk}^{X_r}[n] \end{bmatrix} \quad (4)$$

Then, it follows from the Markovian property that:

$$\mathbf{p}_{X_r}[n+1]=\mathbf{p}_{X_r}[n]\mathbf{A}_{X_rX_r}[n] \quad (5)$$

and therefore:

$$\mathbf{p}_{X_r}[n+1]=\mathbf{p}_{X_r}[0]\prod_{i=0}^{i=n}\mathbf{A}_{X_rX_r}[i] \quad (6)$$

It is straightforward to show that if the individual Markov chains consist of a single recurrent class and if the graph representing the network is acyclic, then the state probabilities will *always* reach a steady-state which can be solved for by propagating the steady-state probabilities of the originating node (the node that is not subject to any influence) into the other nodes. If the graph contains cycles however, a steady-state may not exist. In order to have a full characterization of the network behavior such as the pattern of states that the network can be in at a given point in time, the joint probabilities of state occupancies in different chains is needed. This higher-order analysis is not considered in this paper.

2.2. Related Models

A variety of interacting Markov chains or more generally stochastic cellular automata models have previously been formulated and described, most in the context of studying parallel systems, i.e. systems composed of interacting modules. The most recent of these models and probably the closest to our model are the influence model described in [3] and the stochastic automata network (SAN) in [4]. In a manner similar to ours, both the influence model and the SAN define a network of interacting Markov chains. However both of these models diverge from ours in very important ways. In the influence model [3], the influence from neighboring nodes is constrained to take a multilinear form, while the interaction between Markov chains in our model is clearly non-linear. The difference between our model and the SAN is more subtle. At first glance, it may appear that both models are identical. However, while in our model, the influence is expressed through the *a priori* probability of being in a certain state, in the SAN, the influence is expressed through the *a posteriori* probability of being in a given state. As a result, while it can be shown that the SAN can always be expanded to a higher order Markov chain with much larger state-space, the interacting Markov chain model we present here is not always expandable to a higher order Markov chain. For a more detailed literature review of related models, the reader is referred to [3].

2.3. Cellular signaling

In applying our model to cellular signaling, each protein or enzyme corresponds to a node. The order of the associated Markov chain is given by the number of states the protein can have. For example, a protein can be in one of two states: active or inactive, alternatively it can have three possible states: unphosphorylated, singly phosphorylated, or dually phosphorylated. Non-zero transitions between states are given by our knowledge of the protein state transitions. For example if the protein cannot be dually phosphorylated without first being singly phosphorylated, the corresponding Markov chain will only allow for transitions between the unphosphorylated state and the singly phosphorylated state and between the singly phosphorylated state and the dually phosphorylated state, i.e. no direct transitions between the unphosphorylated state and the dually phosphorylated state are allowed.

Furthermore, when more than one state influences a transition probability in a given node, whether the weighted average model or the fading model is used depends on the actual nature of the protein interaction. Specifically, if the protein interactions are known to be cooperative, i.e. the proteins either bind to the same site or all the proteins in question need to be present to get an activation or an inhibition effect, then the fading model is used. On the other hand, if the proteins bind at different sites or their actions are independent of each other, then the correct model to use is the weighted

average model. Finally, activating interactions are modeled by the positive influence version of $f\{x\}$ and inhibiting interactions are modeled by the negative influence version of $f\{x\}$.

3. A SIGNALING MODULE: THE MAPK CASCADE

3.1. Background

The mitogen-activated protein kinase (MAPK) cascade is an essential component of a wide variety of cell signaling pathways. It is a series of three conserved kinases (i.e. enzymes that add phosphate groups to other enzymes) organized in a hierarchy and found only in eukaryotes. This set of enzymes plays a role in relaying signals from receptors at the cell surface to regulatory elements inside the cell nucleus. They are involved in a variety of pathways ranging from growth, differentiation, and development to inflammation and programmed cell death. At the top of the hierarchy, activated MAPK kinase kinase (or MAP3K*) activates MAPK kinase (MEK) by serially phosphorylating it at two serine residues. Activated MEK then activates MAPK by serially phosphorylating it at a threonine and a tyrosine residue which in turn proceeds to activate downstream signals. The cell also contains phosphatases that dephosphorylate (deactivate) activated kinases. The cascade can thus be perceived as a stand-alone module with the MAP3K activating enzyme as the input signal and the activated MAPK as the output signal.

3.2. Properties of the MAP Kinase Cascade

Many MAP kinase cascade proteins have been studied and quantitative data can be found in the literature for both steady-state behavior [5] [7] and time behavior [6]. Three important properties implemented by this module have been suggested, namely *ultrasensitivity*, *adaptation*, and *bistability*. Probably the most important property is *ultrasensitivity*, i.e. the ability of the cascade to generate a highly switch-like response to a continuously variable stimulus. Specifically, Huang and Ferrell [5] studied the steady-state response of the cascade in Xenopus oocytes and were able to show that in response to a continuous stimulus, the response at the output of the cascade, i.e. the concentration of activated MAP kinase, was more switch-like than at intermediate stages of the cascade such as the concentration of activated MEK. The data is shown in Figure 2. In a parallel and complementary manner, Asthagiri and Lauffenburger studied the MAPK cascade in Chinese Hamster ovary cells [6]. They were able to show that there exists a negative feedback mechanism which leads to *adaptation* of the response, i.e. the time response to a step stimulus generates an output with a peak response followed by an adaptation of the output back to its original value, or close to that value, as if the stimulus was turned back off. The corresponding data is given in Figure 3-(a). Finally, Bagowski and Ferrell [7] suggested that a positive feedback mechanism may occur within the cascade leading to *bistability*: they were able to measure a different steady-state response if the stimulus was stepped up from a low level than if the stimulus was stepped down from a high level as shown in Figure 3-(b). In addition to the experimental findings, a kinetic model based on reaction-rate differential equations was first formulated by Huang and Ferrell in [5]. The simplest model they propose which does not include feedback has at steady-state a total of 25 equations and 17 parameters which need to be estimated. Asthagiri and Lauffenburger in [6] used a similar model with negative feedback. These models were consistent with the data. However, the large number of estimated parameters is problematic.

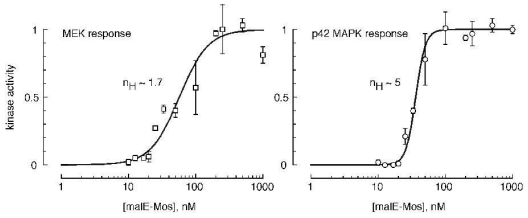
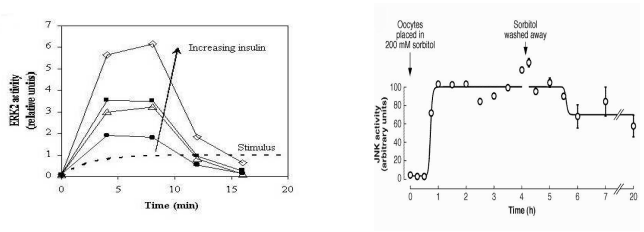


Fig. 2. Experimental stimulus/response data for MEK and MAPK activation in Xenopus oocytes[1]. malE-Mos is the relevant MAP3K in this system.



(a) (b)

Fig. 3. Time evolution data[1]. (a) ERK2 (the relevant MAPK) adaptation in Chinese hamster ovaries. (b) Time course of JNK (MAPK) activation and inactivation in sorbitol-treated Xenopus oocytes. Both insulin and sorbitol are activating factors operating upstream of the cascade.

4. RESULTS

4.1. The Model

Figure 4 shows the interacting Markov chain model of the MAP Kinase cascade. We use the fading version of the model and consider three configurations for the network graph: the open loop case, where all feedback is absent, the negative feedback case where the feedback from state 2 in node x_3 to the transition probability p_{12} in node x_1 is inhibitory, and finally, positive feedback where this same feedback is activating. We also use two different sets of Markov chains at the nodes. In the first set, all Markov chains are two-state chains where one state is the inactive form of the protein and the other state is the active form. A representative chain is given in Figure 4. In the second set, we consider the case of serial phosphorylation where now nodes x_2 and x_3 are represented by three-state Markov chains corresponding to unphosphorylated, singly-phosphorylated, and dually phosphorylated forms of the proteins while node x_1 is still represented by a two-state (inactive and active) Markov chain. Furthermore, the deactivating probabilities are constrained to be equal, i.e. $p_{21}{=}p_{32}{=}p_d$ in all chains where p_d is the common value constant probability. $\alpha_{r,l}$ in equation (1) and $\alpha_{q,p}$ are all set to one for all interactions. $\beta_{r,l}$ are all equal to a constant β, while $\beta_{q,p}{=}0$. Finally, the input signal is $q_{12}^{X_1[n]}$. In the presence of feedback, the feedback interactions are combined with the input (using a value of $\beta_{q,p}{=}1$ and $\alpha_{q,p}{=}1$) using the fading model. Given all these constraints, the model has only two free parameters, namely p_d and β.

[1]Figures 2 and 3 are reproduced with permission of the authors. See [5], [6], [7] for experimental details.

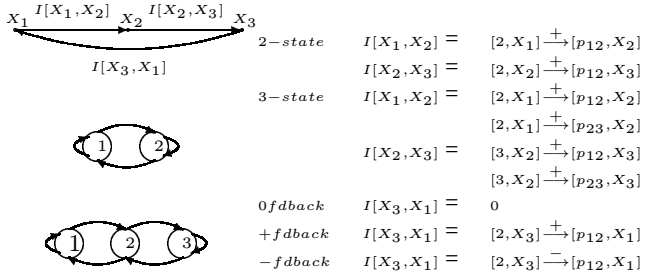


Fig. 4. Interacting Markov chain model of the MAPK cascade. The general network configuration is given as well as the two-state and three-state Markov chains. The influences definitions are also given for all configurations.

4.2. Simulations

The model was implemented in MATLAB and simulations were performed to determine the time behavior as well as the steady-state response using a variety of p_d and β values. For values of p_d in the range [0.05 0.3] and values of β in the range [0.8 3], the shape of the response did not vary much. However, for high values of p_d, the deactivating probabilities dominated the chains and as a result, the probability of being in an active state was very low irrespective of the value of β . The rest of the simulations were therefore performed using the representative values p_d=0.1 and β=3.

4.2.1. Steady-State

Figure 5 shows the steady-state results. For each input value, the corresponding step response was simulated and the steady-state probabilities were recorded. The initial condition for the state probabilities was the value of the steady-state probabilities obtained from the previous run. The experiment was run using increasing input values (from 0 to 1) as well as decreasing input values (from 1 to 0). State zero was initialized at zero.

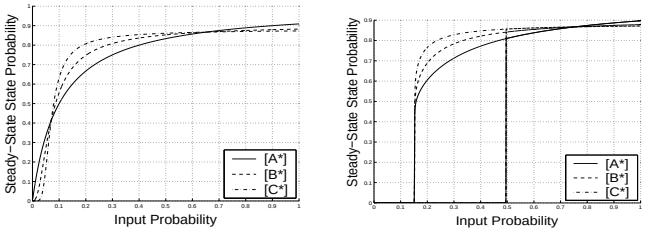


Fig. 5. Steady-state state probabilities as a function of the input probability for the activated states of the proteins. States A^*, B^*, and C^* correspond to activated MAP3K, activated MEK, and activated MAPK respectively.

Figure 5-(a) shows the simulation when no feedback is present and each Markov chain has two states only, inactive and active. It is clear from the figure that as one progresses down the cascade, the response becomes more switch-like. This behavior is much more marked if the serial phosphorylation model with the three-state Markov chains is used (data not shown) which is consistent with Huang and Ferrell's observations [5]. Figure 5-(b) shows the simulations with two-state Markov chains and positive feedback. Clearly, positive feedback makes the response much more switch like. The response also becomes bistable: the curves which sharply change at 0.5 are obtained when the stimulus gradually increases in strength whereas the curves which sharply change at 0.12 are obtained when the stimulus is gradually decreased.

4.2.2. Time Evolution

Temporal dynamics were also examined and the results are shown in Figure 6 for the open-loop and negative feedback cases. The time response was computed following a step input of magnitude 0.6 at time zero. The results show that adaptation is observed only when negative feedback is present as measured by Asthagiri and Lauffenburger [6].

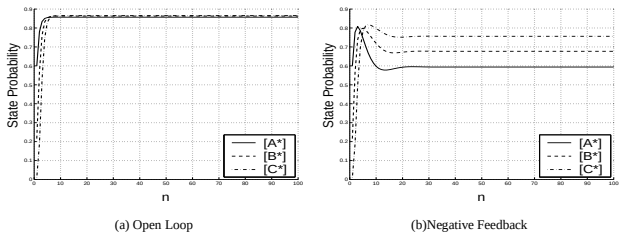


Fig. 6. State occupancy probabilities as a function of n. A^*, B^*, and C^* are defined as in Figure 5

5. SUMMARY

In this paper, we presented an approach to modeling signal processing in cells based on interacting Markov chains. The example of an evolutionary conserved signaling module, the mitogen-activated protein kinase pathway, was given and it was shown that the model correlates with experimental findings. Specifically, ultrasensitivity, adaptation, and bistability were observed. Compared to other modeling approaches such as biochemical reaction-rate models, the model presented here has very few parameters and its dynamic properties are a consequence of the topology of the network as opposed to some particular set of parameter values.

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