

A functional representation of the simulation data of biochemical models based on molecular activity

SIMON HARDY, PIERRE N. ROBILLARD

Département de génie informatique et génie logiciel

École Polytechnique de Montréal

C.P. 6079, succ. Centre-Ville, Montréal (Québec) H3C 3A7

CANADA

simon.hardy@mssm.edu, pierre-n.robillard@polymtl.ca

Abstract: - Interpreting the simulation data of a complex biochemical model to understand its dynamic behavior is a difficult task. Traditional data representations display simulation outputs as concentration plots. To study the dynamic behavior of a model from these plots, it is necessary to have in mind the topology of the modeled system, know the function of the individual elements of the system and be able to describe their activity. Only with this mental image of the model can the dynamic behavior be deciphered. In this paper, we suggest exploiting this knowledge to create a preprocessing filter for the simulation data. This data filter is based on the concept of molecular activity and transforms the simulation data from a concentration perspective to a molecular activity perspective. This is done in two steps: identify the functional groups of the system, and mathematically describe the molecular activity of these groups. In this paper, we demonstrate this new data representation approach with a complex model of the signal transduction system of long-term potentiation in the hippocampal post-synapse, a model exhibiting a bistable behavior. To facilitate viewing of the resulting data matrix, the preprocessed data are displayed with known visualization techniques, followed by the production of an animated and a spectral functional representation. One advantage of the functional data filter is that, once created, it can be applied to a large number of simulation runs while at the same time performing parametric and structural modifications on the model in order to quickly explore the impacts on the model's behavior.

Key-Words: - biochemical modeling, simulation data, system dynamics, function, visualization

1 Introduction

The simulation of biochemical models based on kinetic reactions generates data time series of concentration. To interpret these time series, the data are usually displayed on Cartesian graphs where time is the x-axis and concentration the y-axis. These plotted data are used to study the dynamic behavior of simulated models by combining sets of graphs. This simple type of representation is convenient for the study of simple models, but using it to study the dynamic behavior of complex models is a difficult task. Biochemical systems are highly organized and composed of many heterogeneous processes, formed by the interaction of the activities of different molecules. The signaling pathways of the cell are typical cases of such processes, where signals are transmitted by the perturbation of enzymatic activities and the actions of molecular messengers. An example of enzymatic activity is phosphorylation: some proteins are turned "on" and "off" by the addition and removal of a phosphate group, catalyzed by a

kinase. Thus, it is possible to link a structural modification of a molecule to its role in a biochemical process.

Researchers develop models of signaling pathway networks on the basis of experimentally established relationships between molecules, their function and the property of a biological system under study. Analyses of the simulation data of these models require a deep understanding of the underlying molecular or biochemical processes. To make sense of the simulation data, they are processed and filtered according to the researcher's understanding of the activities of the system components. Our analysis will focus mostly on some relevant dynamic behaviors of model components.

The original approach presented in this paper enables researchers to explore a new functional interpretation of a signaling pathway based on their existing knowledge of molecular activities by using simulation data. This functional interpretation transforms the data from a molecular concentration

perspective to a molecular activity perspective, a transformation which is achieved in two steps. In the first step, the molecular substances of the model are grouped into functional units, a functional unit being defined as a group of molecules participating in a given activity performed by the signaling network. In the second step, the activity of each functional unit is mathematically formalized by an equation. This equation defines an indicator of the degree of molecular activity as a function of the concentration of the molecules of a functional unit. The parameters of the equation are empirically defined by the researchers based on their understanding of the molecular processes at the functional unit level.

This new functional interpretation of the simulation data provides a unique dynamic perspective when combined with powerful visualization techniques. Colors are used to represent the degree of molecular activity of the functional units of a model. The mapping of these colors on a graph displaying the topology of the model creates a highly informative animated graphical representation of the dynamic behavior of the model.

This paper presents the use of this functional interpretation approach combined with visualization techniques based on the simulation data of the model of the signal transduction system of long-term potentiation (LTP) in the hippocampal post-synapse. Bhalla and Iyengar [1] were the first to model this network. Their model was later modified by Kikuchi *et al.* [2]. This system is interesting because of its property of bistability, which, it is hypothesized, is linked to the neuronal memory processes. Representation of the data from a functional perspective clearly illustrates this systemic property, as well as the dynamic behavior of the system and its molecular components.

2 Previous work

In many different scientific fields, making sense of large quantities of complex data can be perilous without the help of data processing and visualization techniques. This is the case for genome, transcriptome and microarray data, protein interaction maps and metabolic pathways – molecular biology is no exception. Biologists use data analysis and visualization techniques to both explore their data and present their results. In computational biology, the simulation of complex biochemical models can also generate a substantial amount of data. The interpretation of this data can be a slow and inefficient process using the

traditional means of data representation: two-dimensional plots of concentration-time series data. Another problem with the presentation of the simulation data of biochemical models with raw numbers and plots is the loss of the network topology, which is information that is crucial to building a mental representation of the dynamic behavior of a model.

Prior to the creation of the molecular activity perspective developed in this paper, software tools had been developed to try to solve these problems. MetVis [3], SimWiz [4] and BioPathwize [5] make it possible to visualize the simulation data of metabolic networks and signaling pathways in a more comprehensive way. These tools show a manually or automatically generated network graph to which the simulation data are mapped. This creates an animated representation of the data. Either the edges or the nodes of the network graph change in color or volume to show the evolution of the concentration of the substances making up the biochemical network. The tools MetVis and SimWiz are also available in a three-dimensional version [6][7].

The advantages of the animated views produced by these tools are numerous. They take advantage of the benefits of visual data exploration, one of which is to integrate the human perceptual abilities into data interpretation, a useful asset in the exploratory steps of data analysis [8]. They also make full use of the capacity of the human mind to detect structures and patterns in images, such as synchronicities, global changes of state and oscillations [9]. The developers of MetVis and SimWiz stress the usefulness of the topology of the biochemical networks for their animated graphical representations. To map the simulation data to the model structure gives a good impression of the dynamic behavior of the system. Thus, a wealth of information becomes accessible to the user.

Despite these advantages, the animated representations generated by the existing visualization software tools of the biochemical network simulation data show only the variation in concentration of the network substances. In short, they provide a single point of view without any data processing, which is the concentration perspective. The same is true for concentration plots. These tools fail, however, to fully benefit from another data analysis method: the visualization of data with multiple views [10]. By displaying data in multiple ways, the user may interpret the data through different perspectives, hasten its understanding and

avoid possible misinterpretations. Visualization systems usually follow a four-step pipeline dataflow model: the data are filtered to create a subset of data, which is then mapped to a representation which can be displayed. To produce multiple views from the same data set, three modifications of the visualization dataflow are possible: 1) modification of the data filter; 2) modification of the data mapping; and 3) modification of the display. A modification of the data mapping or the display, as proposed by the tools MetVis and SimWiz with the transformation of concentration plots into a topological view, preserves the same data filter: concentration variation. The functional perspective presented in this paper is based on a modification of the data filter, which is the first step in the pipeline dataflow model.

3 The long-term potentiation signal transduction model

Much research has been conducted on the *synaptic plasticity* of the neuron. This property enables the neuron to undergo a lasting alteration to the efficiency of its neurotransmission signaling process. When there is an enduring increase in the amplitude of post-synaptic exciter potentials, synaptic plasticity is called *long-term potentiation* (LTP). LTP was first described by Bliss and Lømo [11]. Experimentally, it is caused by series of short, high-frequency electrical stimulations, also described as *tetanic stimulations*, to a nerve cell synapse which strengthen, or potentiate, that synapse for minutes or hours. Research on LTP (and its opposite, *long-term depression* or LTD) is in part motivated by the assumption that synaptic plasticity forms the cellular basis of learning and memory. [12] is an introductory paper on LTP, and [13] is a review of the theoretical and experimental research on synaptic plasticity. Many kinetic simulations of LTP at the molecular level, as well as models based on phenomenological facts, have been developed to theoretically study this complex property. As reported in a previous paper, we transformed these two kinds of model into a single, Petri net-based representation [14].

Bhalla and Iyengar [1] developed a complex LTP model at the molecular level composed of many simple networks combined to form post-synaptic signaling pathways. A remarkable aspect of their work is that they used a systemic approach to unravel long-term potentiation. They demonstrated

that their model was characterized by the property of bistability, as a result of the combination of multiple networks, since none of them exhibited this behavior by itself. They based their work on the experimentally observed fact that bistability is an enduring biochemical modification linked to LTP. New experimental evidence has led Kikuchi *et al.* [2] to add some reactions to this model, involving the dynamic inactivation of the protein phosphatase 2A (PP2A). This addition was significant because PP2A affects important molecules in synaptic plasticity, and thus has an impact on the bistability of the system. Adding the dynamic modeling of PP2A made the LTP model more robust and facilitated LTP induction in the system. The block diagram of the modified model of the signal transduction system of LTP is shown in Fig. 1. In this figure, rounded rectangles represent enzymes, circles represent messenger molecules, rectangles represent receptors and dotted, rounded rectangles represent a reaction module. Regular arrows represent activation and circle-ended arrows represent inhibition. Each block in this diagram corresponds to a set of chemical reactions between several substances. Consequently, the blocks are simplifications of more complex networks.

In the remainder of this paper, the simple interconnected networks of the model are referred to as *modules*. The modules of the LTP model are represented in two different ways in Fig. 1: either a module is composed of a number of blocks (in which case, the module is represented by a dotted, rounded rectangle) or a module is a single block outside a dotted rounded rectangle (like PKA, CaM and CaMKII). One module is a set of chemical reactions involving approximately five to fifteen substances, and each reaction has constants, all of which have been experimentally obtained and documented. Those parameters are compiled in the DOQCS database [15]. The model contains approximately 200 substances and 350 reactions. The reader is referred to Kikuchi *et al.* [2] for more information about the model.

The model was simulated with the software E-CELL [16]. Model bistability from a normal steady state to a potentiated steady state was observed by inducing a tetanic inflow of calcium ions to the model. The entry point of the model is calcium, and the increase in its concentration activates several enzymes, which in turn activate various molecules. The activity of the model's interconnected positive and negative feedback loops, combined with the

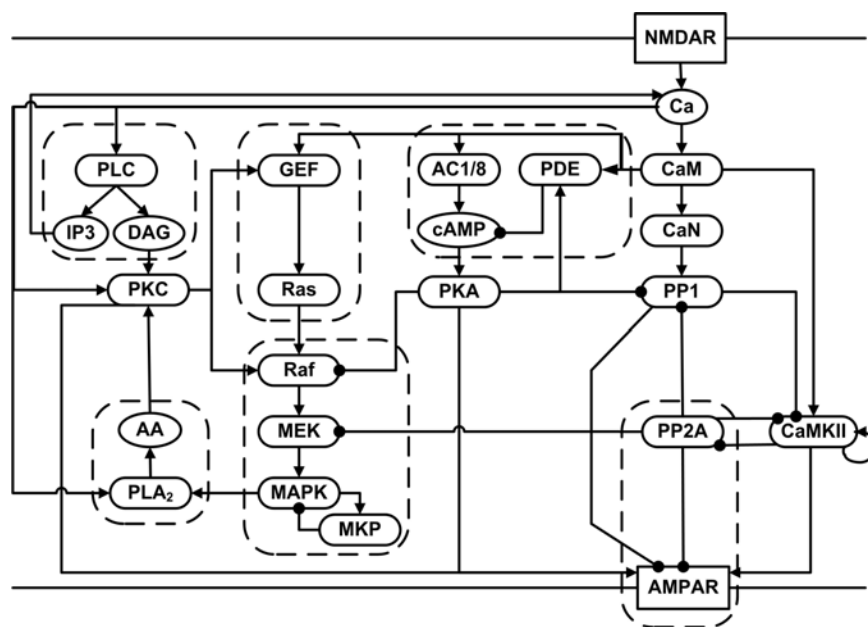


Fig. 1. Block diagram of the long-term potentiation (LTP) signal transduction system in the hippocampal post-synapse.

appropriate stimulus, leads to a potentiated steady state.

4 Creation of a molecular activity perspective of the simulation data of the LTP model

The first step in the transformation of the simulation data from a concentration perspective to a molecular activity perspective is the identification of the functional units of the LTP model. Grouping the substances of the LTP model into functional units was performed by interpreting the reaction equations of the modules and the topology of the model, with the experience gained with the model and according to engineering reasoning. A key factor in the identification of the functional units of the LTP model is to group together the different configurations of a molecular species (for example, the calmodulin (CaM) functional unit is composed of the following different molecular configurations of the CaM molecules: CaM, CaM.Ca₂, CaM.Ca₃, CaM.Ca₄, CaM.Ng). However, the functional units are specific to the biological process under study and cannot be derived from formal analysis of the model. This is discussed in more detail later in this paper.

Fig. 2 shows examples of functional units. In this case, the PKA module of the LTP model is divided into the following three functional units: 1) the R₂C₂

complex unit, where the two R complex subunits first bond successively to four cAMP molecules, after which the complex releases two PKA enzymes; 2) the PKA inhibitor unit, in which inhibitor molecules are either in a free or a coupled configuration with inhibited PKA enzymes; and 3) the PKA enzyme unit. These units represent the three main activities performed by the molecules of the PKA module, which are, respectively, the entry point of the module, where a molecular complex needs input molecules to produce an important enzyme; the inner control mechanism; and the output molecule that will interact with other modules. One of the advantages of forming functional units is to reduce the number of numerical values of interest. In the PKA module, ten concentration values are expressed with the activity level value of three functional units. For the entire LTP model, 200 concentration values are expressed with an activity level value of 34 functional units.

The second step in the transformation of the simulation data is to mathematically describe the activity of each functional unit by an equation. The continuous concentrations of the substances of the functional unit are converted into discrete states indicating the level of activity of the functional unit. This conversion is performed with equations using concentrations as inputs to compute a value on a discrete scale from 0 to 10 (0 being the lowest level of activity for a functional unit, 10 being the

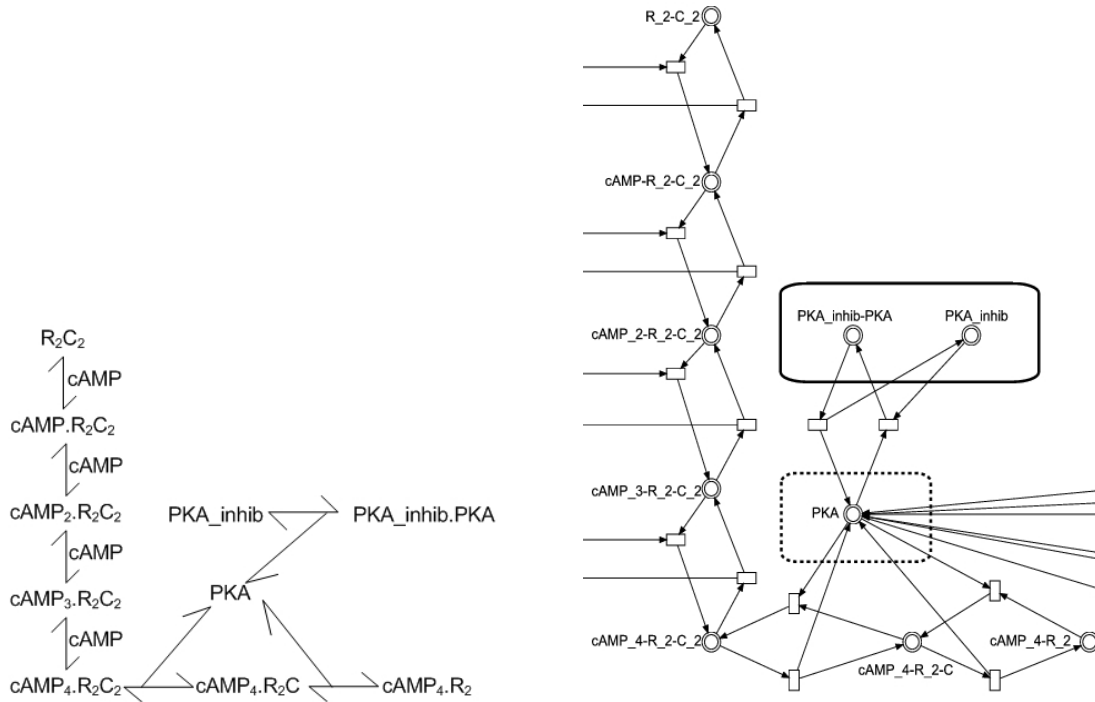


Fig. 2. Reaction equations and functional units of the protein kinase A (PKA) module of the LTP model (see Fig. 1). On the left is the reaction scheme of the PKA module. On the right is the same reaction scheme represented in a hybrid functional Petri net notation, where double circles are substances, rectangles are reactions and arrows indicate the direction of the reaction (see [17] for more information about Petri nets and molecular biology). The PKA module is divided into three functional units: one grouping together the various configurations of the R_2C_2 complex with cAMP molecules, one for the PKA enzyme itself and one grouping together the PKA inhibitor and the complex formed with the inhibited PKA and the inhibitor.

highest). For the LTP model, each functional unit has a state equation designed to produce an activity level value that is consistent with the unit behavior. Equations 1, 2 and 3 are the equations of the R_2C_2 complex unit, the PKA inhibitor unit and the PKA enzyme unit of the PKA module. The equations are used to determine the activity level of the three functional units at every simulation time step.

$$[state_{R_2C_2}] = \frac{10}{50} \cdot \left(\begin{array}{l} \frac{0}{6} \cdot [R_2C_2] + \frac{1}{6} \cdot [cAMP.R_2C_2] + \\ \frac{2}{6} \cdot [cAMP_2.R_2C_2] + \frac{3}{6} \cdot [cAMP_3.R_2C_2] + \\ \frac{4}{6} \cdot [cAMP_4.R_2C_2] + \frac{5}{6} \cdot [cAMP_4.R_2C] + \\ \frac{6}{6} \cdot [cAMP_4.R_2] \end{array} \right) \quad (1)$$

$$|state_{PKA_{inhib}}| = 20 \cdot (0,5 - [PKA_{inhib}]) \quad (2)$$

$$[state_{PKA}] = 100 \cdot [PKA] \quad (3)$$

For the purposes of this paper and to explain the functional representation, we provide a general description of the design of the first state equation.

Equation 1 uses the concentration of the seven molecular configurations of the R_2C_2 complex. The function of this unit is to produce PKA enzymes when cAMP molecules are available in sufficient quantity. In its least active configuration (R_2C_2), the R_2C_2 complex is not bound to any cAMP molecule. In its most active configuration ($cAMP_4.R_2$), it is bound to four cAMP molecules and has released two PKA enzymes. The other five molecular configurations can be positioned relative to this activity scale. According to the distribution of the concentration of the R_2C_2 complex in the seven configurations, the equation gives a value from 0 to 10, associating a discrete value to the level of the activity of the functional unit. If all the complexes are in the R_2C_2 configuration, the level of activity is 0. If all the complexes are in the $cAMP_4.R_2$ configuration, the level of activity is 10. If the complexes are distributed among the seven configurations, the level of activity will be between 0 and 10. The first constant of equation 1, $10/50$, is a normalization constant, 50 being the total

concentration for every R_2C_2 configuration, and 10 being the number of possible activity levels.

5 Visualization of the simulation data from a functional perspective

The 34 state equations of the LTP model each provide an integer from 0 to 10 for every time step of a simulation run. An easy way to visualize this data matrix is to associate the integer values with colors. For the LTP model, we used the colors of the light spectrum. Violet is associated with the state 0, low activity, and red is associated with the state 10, high activity. The color association process is complete when the color of every functional unit for every simulation step is determined. Subsequently, the colored functional data are displayed in two different types of visual representation. The first representation is an animated, global view of the model, where the colored functional data are mapped to the topology of the model. The result is that the variation in the activity of all the model components can be seen simultaneously. This view is made up of a succession of images of the colored functional units of the model for various time steps, in other words an *animation*. The second representation is a collection of spectra of the functional units, showing their changing level of activity through time. A *spectrum* is a timeline, where the color changes represent the activity variation for a single functional unit.

The next two subsections present the animated and spectral representations of two simulation runs of the LTP model. The first simulation run is based on normal concentrations and parameters. For the second simulation run, the value of the K_{cat} parameter of the inactivation reaction of PP2A by CaMKII.CaM is modified. The normal value of the parameter is 5 s^{-1} and the modified value is 0.5 s^{-1} . These values were extracted from the work of Kikuchi *et al.* (2003). The modification is known to affect the model's bistability. In both simulation runs, a tetanic calcium stimulation is induced at 120 seconds. The concentration of the 200 substances of the model is recorded at every second. The simulation duration is 1,000 seconds. The simulation outputs, a set of the concentration data of the 200 substances for each simulation run, were transformed to create a colored functional perspective.

5.1 Animated functional representation of simulation data of the LTP model

The animated functional representation of the simulation data of the LTP model is a mapping of the colored functional data to a graphical representation of the topology of the signaling network. The graphical representation of the topology is a graph where the functional units are displayed as nodes. Regular and rounded arrows link the functional units to show the activation and inhibition relationships. The nodes are colored according to their level of activity computed from the simulation data. A change in color reflects a change in activity level. The animated representation of the first and second simulation runs can be viewed at the website [18]. Four frames of the animation of the first simulation run are shown in Fig. 3. Two frames of the animation of the second simulation with the modified parameter are shown in Fig. 4. Subfigure 3A shows the system from the functional perspective of both simulation runs at 90 s (normal steady state), subfigure 3B shows the system at 124 s (a few seconds after the tetanic stimulation), subfigures 3C and 4A show the system at 200 s (in transition to the final steady state) and subfigures 3D and 4B show the system at 1,000 s (almost final steady state).

An initial visual exploration of subfigure 3A shows mainly cold colors. Before the introduction of any calcium input, the system is in a non-potentiated state. Only the PP1 functional unit is active, as its red color indicates. The PP1 functional unit has a unique behavior in the LTP model, in that it is the only unit with a reverse behavior: at the normal steady state, the PP1 functional unit is highly active, while at the potentiation steady state it is inactive. The function of this unit is inhibitory; it limits the activation of the CaMKII and PP2A units. Part of the system bistability property is triggered by a persistent diminution in the PP1 unit inhibition activity, in order to let the CaMKII and PP2A units be persistently more active.

In subfigure 3B, 4 seconds after the induction of the calcium stimulation, the cyan color of the calcium functional unit (Ca) denotes the higher concentration of this ion due to the tetanic calcium stimulation. The modules directly connected to the Ca unit, such as the PLC, PLA₂, CaM and CaN modules, react rapidly. The change in color of the first-degree neighbors of the Ca unit shows an activity increase, which has also been communicated to some of the Ca unit's second-degree neighbors, the GEF and cAMP modules.

Subfigure 3C shows the state of the system 80 seconds after the Ca stimulation. Two groups of functional units behave in distinctive ways. The first-degree neighbors of the Ca unit are returning to their initial state. However, the modules at the end of the signaling network, such as the MAPK, PP1, CaMKII and PP2A modules, which are slower to react to the Ca stimulation, are showing an increase in their activity.

This transitional state leads to the final state of the system, 880 seconds after the Ca stimulation and shown in subfigure 3D. First-degree neighbors of the Ca unit are back in their initial state. The final colors of the modules at the end of the signaling pathway, however, which are located at the bottom of the frame, indicate a persistent activity in the system's final state. This is the potentiated state. Subfigures 3A and 3D are visual representations of the bistability of the LTP model. According to Kikuchi *et al.* [2], a particularly important functional unit is the AMPAR unit (at the bottom center of the model). At the beginning of the simulation, this receptor is in a depressed, or inactive, state (blue), and, at the end of the simulation, this receptor is in a potentiated, or active, state (orange).

The visual exploration in Fig. 4 shows a different behavior of the LTP model. The frames of the simulation of the modified model at 90 s and 124 s are not shown because they are identical to the same frames of the normal model. This suggests that the initial state and the first reactions to the Ca stimulation are similar in the two versions of the model. The impact of the modified parameter appears later in the simulation. Subfigure 4A shows a different transitional state: the modules at the end of the signaling pathway are less active than they were in Fig. 3C. The last frame of the animation, shown in Subfigure 4B, displays a final state that is similar to the initial state. Thus, this version of the LTP model is not bistable.

5.2 Animated functional representation of simulation data of the LTP model

The animated representation is made up of a sequence of images of the network topology showing the activity level of every functional unit of the model as time progresses. It enables analysis of the system behavior and provides information on the interactions between modules. The spectral representation is the set of colored timelines of the

level of activity of the functional units. It provides a view of the complete simulation period. Fig. 5 shows the spectral representation of the functional units for the simulation data of the LTP model.

Fig. 5 shows the spectral representation of the functional units of the modules exhibiting a modification in their level of activity. A spectral representation can display more information in less space than a traditional representation using charts and plots. The spectra of the CaM and the CaN functional units for the two simulations confirm the behavior of the first-degree neighbors of the Ca unit suggested by the animations. They react rapidly to the increase in the calcium concentration, and their activity pattern is, for the most part, unaffected by modification of the PP2A reaction parameter. Because their final state is the same as their initial state, these units do not exhibit bistability. This observation is also true for the functional units of the PLC and cAMP modules. The spectra of the MAPK, CaMKII, AMPAR and PP2A functional units illustrate the behavior of the units at the end of the signaling network. Their activation is slower than the activation of the Ca first-degree neighbors, and it occurs after a delay. The activity pattern of the spectra of these units, produced from the simulation of the normal LTP model, shows the bistable property of this network: from an inactive, initial state, the spectra indicate that the functional units stabilize to a different and more active state. On the spectra of the modified LTP model, we can see a different activation pattern, one without bistability. These three functional units, after a transitory change in their activity level, eventually return to their normal steady state after the Ca stimulation. As already explained, the PP1 functional unit exhibits a reverse activity relative to the other functional units of the system. The MAPK, CaMKII, AMPAR and PP2A units change from a low level of activity (cold colors) to a higher level of activity (warmer colors) as the PP1 unit undergoes the opposite variation.

The spectra of the PLA₂ and AA functional units are examples of a combined behavior involving the behavior of a Ca first-degree neighbor and that of an end-of-signal unit. An initial activity change to the stimulus induction is noticeable immediately after this, followed by a return to its original, inactive state. A second inactivation occurs later, resulting in a persistent activation of the unit in the normal model.

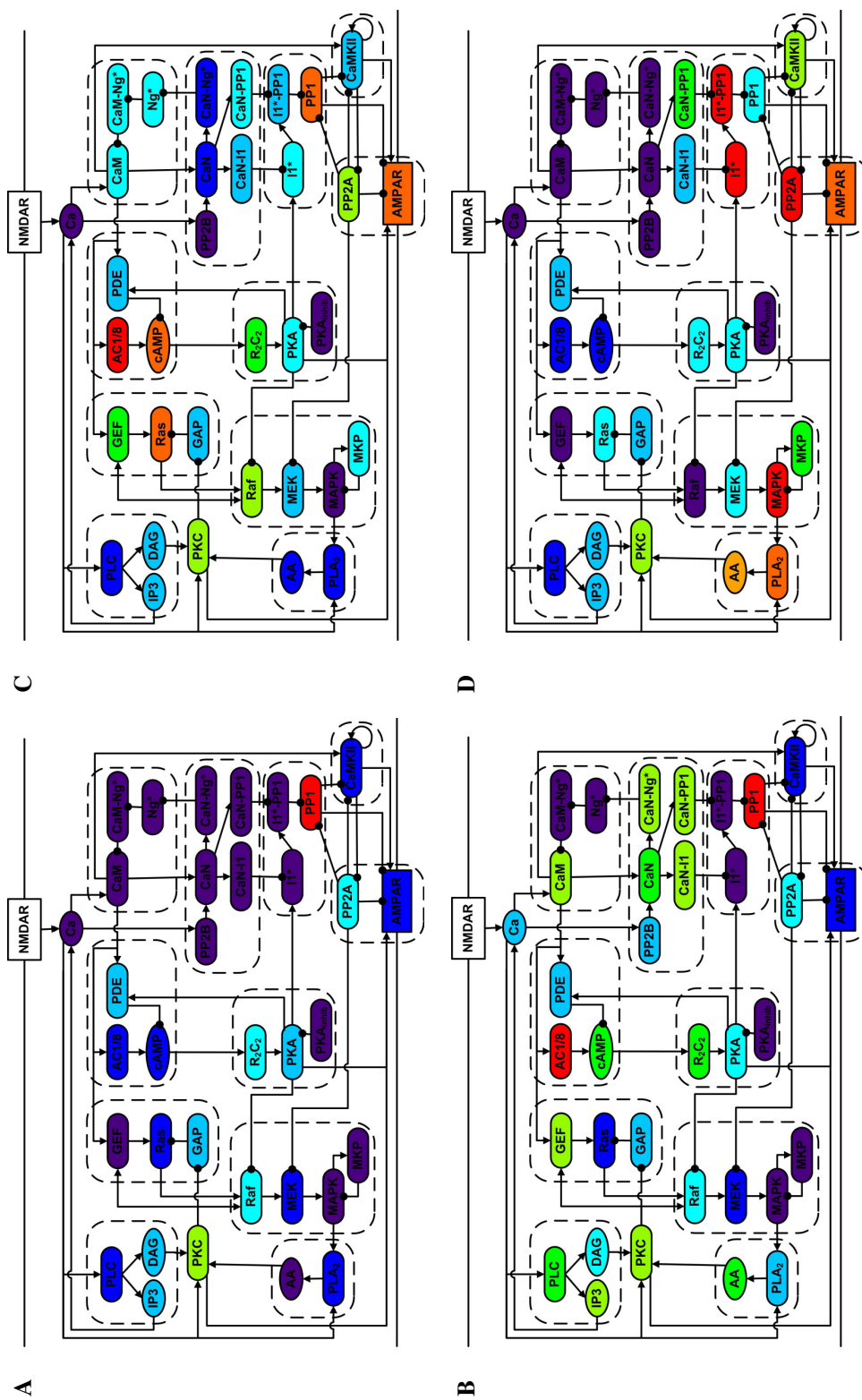


Fig. 3. Four frames of the animated representation of the LTP model simulation in normal conditions. Subfigure A is a representation at simulation time 90 s, subfigure B is at simulation time 124 s, subfigure C is at simulation time 200 s and subfigure D is at simulation time 1,000 s.

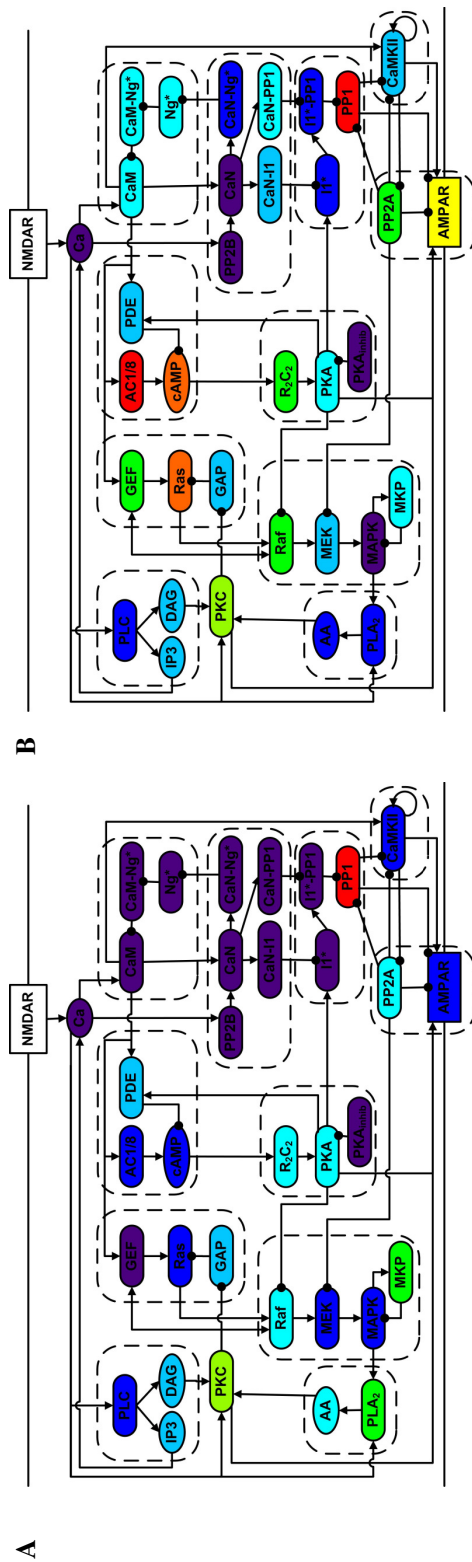


Fig. 4. Two frames of the animation of the LTP model simulation with the parameter of the inactivation reaction of PP2A by CaMKII modified. Subfigure A is at simulation time 200 s and subfigure B is at simulation time 1,000 s.

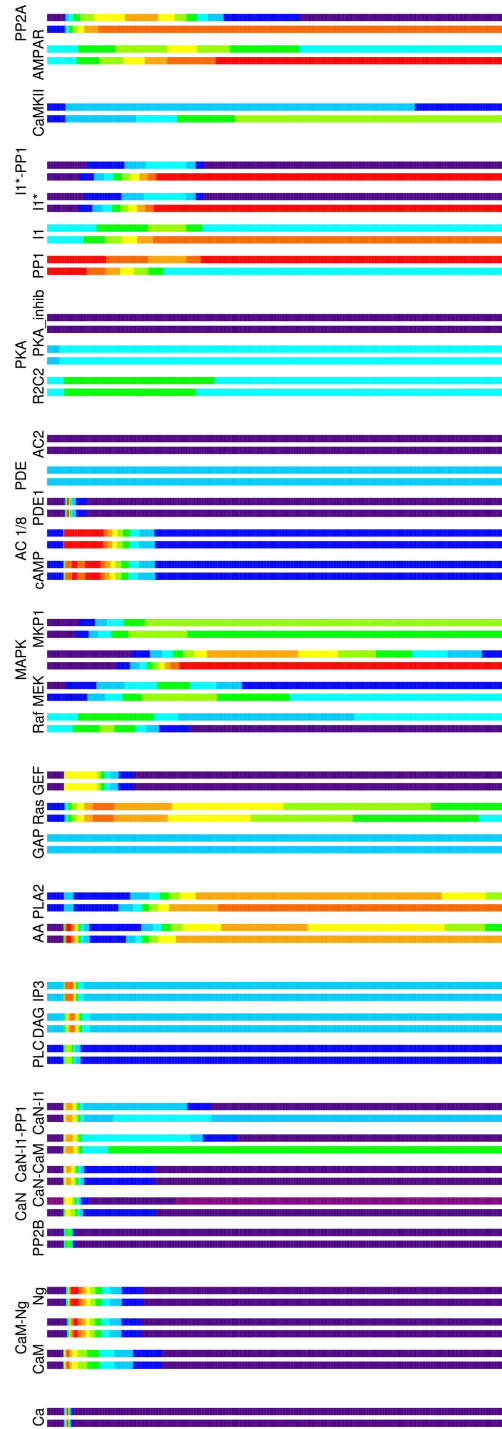


Fig. 5. Spectral representation of the functional units of the LTP model. For each unit, the spectrum on the left is the visualization of the simulation outputs of the normal LTP model, and the spectrum on the right is the visualization of the simulation outputs of the modified LTP model. The Ca spectra indicate the calcium stimulus at 120s. The activity-level scale is at the left (violet = inactivity, red = activity) and the time proceeds from top to bottom, starting at 90 s and ending at 1,000 s.

6 Concluding remarks

In this paper, we explored an innovative perspective of the simulation data: a new data filter to present and analyze the data, based on the concept of molecular activity. This data filter is an approach to creating data subsets: functional units. In two steps – identification of the functional units and mathematical description of the molecular activity of the functional units – the simulation data are transformed, with a functional data filter, into data on the molecular activity of the model. This perspective of the molecular activity of the simulation data of a biochemical model formed the basis for two views of the simulation data of the model: a spectral functional view displaying the behavior of individual functional units, and an animated functional view of all the functional units of the model. This last type of data representation integrates the system structure and its dynamics into a single view. One of the goals of these views is to represent many numerical simulation outputs in such a way as to more easily access a system's overall systemic behaviors. This is achieved by identifying the functional units of a system and by visualizing the interactions between them. As demonstrated in this paper, this approach can replace numerous traditional concentration graphs and will help enable the rapid observation of the impacts of structural or parametrical modifications of the model. This is an asset for the computational biologist developing a biochemical model. With the molecular activity approach, the computational biologist can create a data-preprocessing filter and afterwards easily explore the parameter space of the model and study the resulting behavioral modifications. This filter, built with the knowledge he or she usually uses to interpret the raw simulation data, is implemented only once and can then be applied to the data of any number of subsequent simulation runs. In this way, the modeler will have rapid access to the dynamic behavior of the different instantiations of a model.

A limitation of the simulation data representation approach based on molecular activity is that it is only efficient for qualitative analyses. The level of molecular activity is assigned on a relative basis that is specific to the characteristics of each functional unit. The result is a qualitative indication of the activity level of a functional unit. Despite this limitation, a qualitative glance at the dynamic behavior of a model is useful in the initial exploratory steps of simulation data analysis, while concentration graphs remain useful for detailed quantitative analyses. Taken together, concentration

graphs and a data representation based on molecular activity are complementary approaches constituting two distinct perspectives, and it is advantageous to use them together.

At the moment, our approach and its two steps cannot be implemented in a systematic method for two reasons. First, the composition of functional units is intended to be customizable in order to fit research hypotheses and goals. The composition of functional units can be adapted to fit either micro- or macrosystem dynamics, thus allowing the representation of a model behavior at different levels of abstraction. The composition can also be focused on some specific molecular activities and leave some other activities out. In the LTP model, this is exemplified with the calcineurin module (CaN). If the criterion for the composition of the functional units of this module was to be solely based on the recognition of a single molecular species and its various configurations, only one functional unit would be identified for this module. However, this protein phosphatase has three distinct regulatory effects of dephosphorylation on neurogranin (Ng) and inhibitory-1 protein (I1). To distinguish among these three different molecular activities, three functional units were identified, one for each dephosphorylation activity. The second reason why this data representation approach based on molecular activity cannot be implemented in a systematic method is that, as we have already mentioned, it does not involve a specific modeling language. Each modeling language will require its own algorithm. Depending on whether the model is specified with ordinary differential equations, is a Petri net model or has a stochastic nature, the algorithms will be different because of the particularities of the languages. However, the properties of a modeling language can be useful for the development of such an algorithm. Part of our current work involves the use of the invariants of a Petri net, which are structural properties, to implement an algorithm.

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