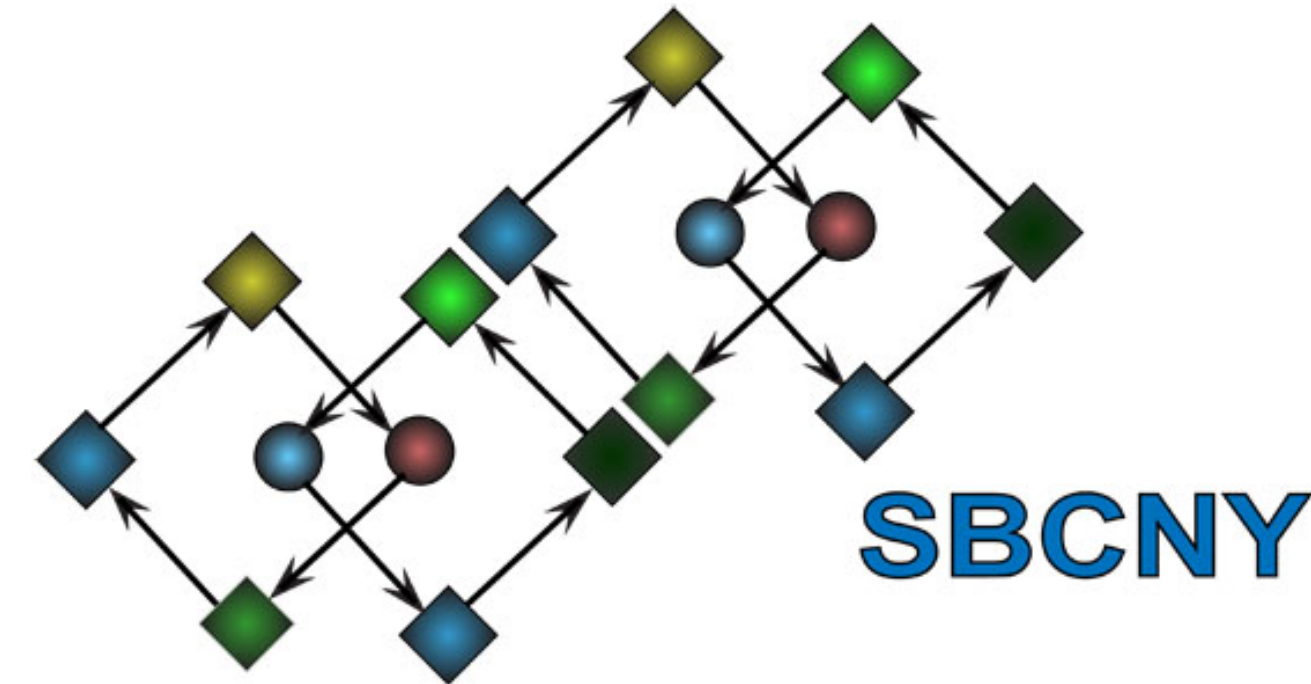




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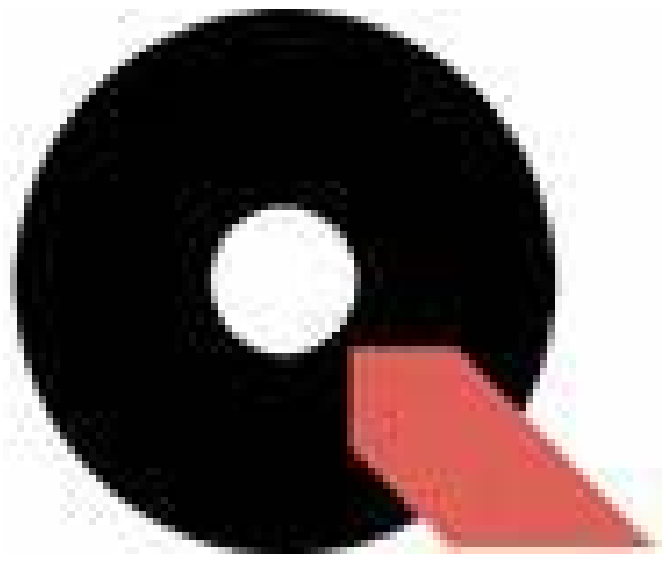


# Analysis of Regulatory Motifs Dynamics in Cellular Signaling Kinetic Models

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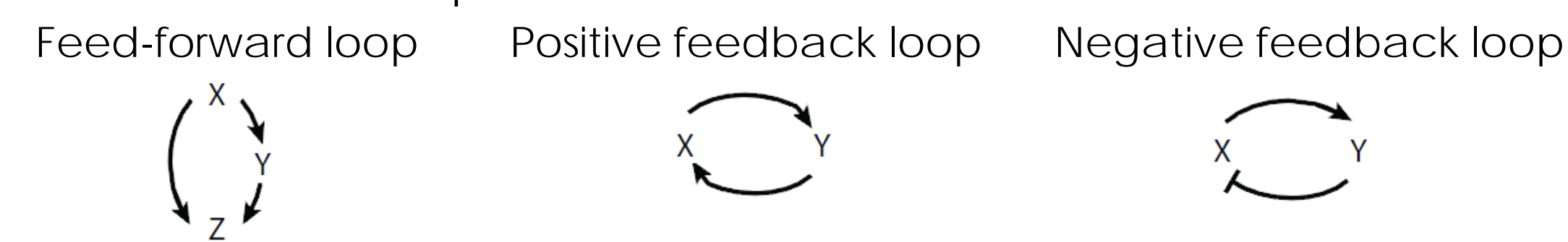


## Abstract

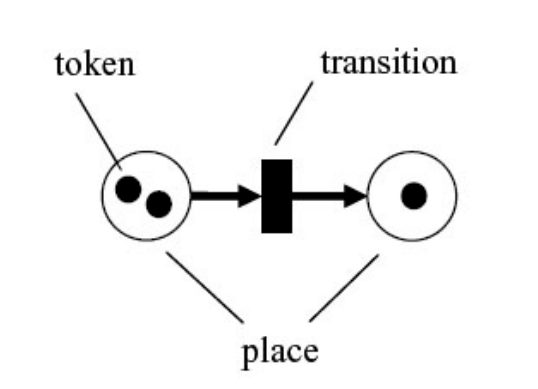
The cell uses complex networks of interacting components in order to process information in response to extracellular and intracellular environmental changes. These networks are made up of genes and proteins interacting in regulatory motifs. Regulatory motifs can be defined as simple patterns of activation and inhibition, such as positive and negative feedback loops. The dynamics of regulatory motifs are commonly modeled and analyzed with differential equations, but the topology of the interactions may not be explicit from the calculations. To create a more intuitive representation of how cellular components interact, we use Petri nets to build dynamic graphs to represent the dynamics of regulatory motifs. We create the interaction network representation of an ODE model, divide it into its topological signaling components and animate it with concentration and flux values in order to construct a dynamic network representation of numerical simulation data. We demonstrate the utility of this method using two established models of cellular regulatory systems: a model of the cell cycle (1) and a model of  $Ca^{2+}$ /calmodulin-dependent protein kinase II (2). Results with the first model show that the arrangement of cyclin-dependent kinases in feed-forward loops (FFLs) allows for these loops to activate different proteins at different phases of the cell cycle depending on the effect of the regulatory steps of the FFL (+ or -). In the second system, we explore the interaction of multiple signaling pathways to form networks that have properties that individual pathways do not. A dynamic representation of the CaMKII network shows that bistability and sustained CAMKII activity is due to the interaction of the CAMKII pathway with the cAMP pathway and with a positive PKC-MAPK feedback loop within a nested feed-forward motif.

## Background

The cell's information-processing abilities involve the use of complex networks that can be broken down into regulatory motifs. These motifs carry out specific functions in the cell. Such functional motifs include feedforward loops and feedback loops

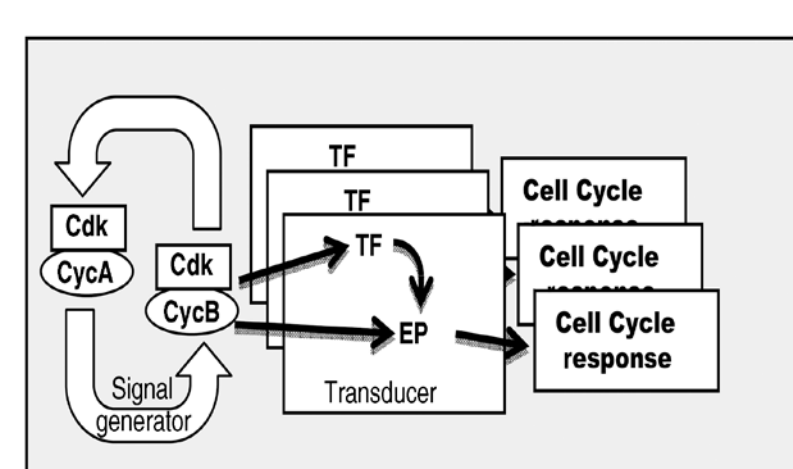


Petri nets can be used to emphasize the dynamic of regulatory motifs. Petri Net formalism is a mathematical modeling tool to represent causal systems. Petri nets consist of places, transitions, and arcs. Places can contain tokens, or markings that represent the current state of a system, and transitions represent activities which can occur. Places and transitions can be associated with variables and rates from an ODE model.

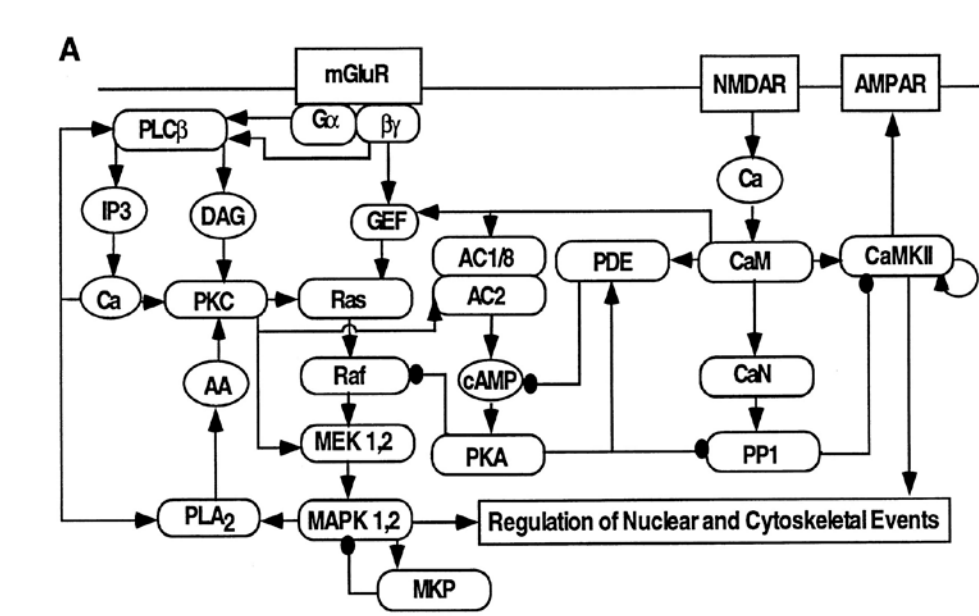


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A eukaryotic cell's progression through the cell replication division cycle is regulated by oscillations in CDKs. When activated by bound cyclin, CDKs directly phosphorylate executor proteins (EP) or phosphorylate transcription factors (TF) that control the expression of EPs, thereby forming a feed-forward loop.



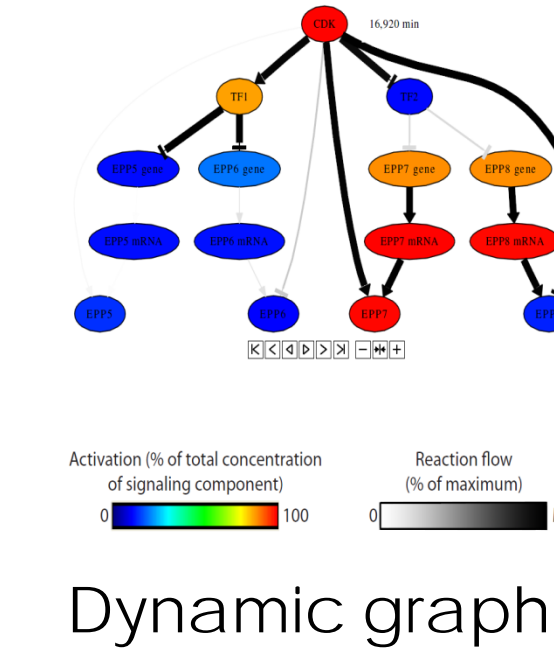
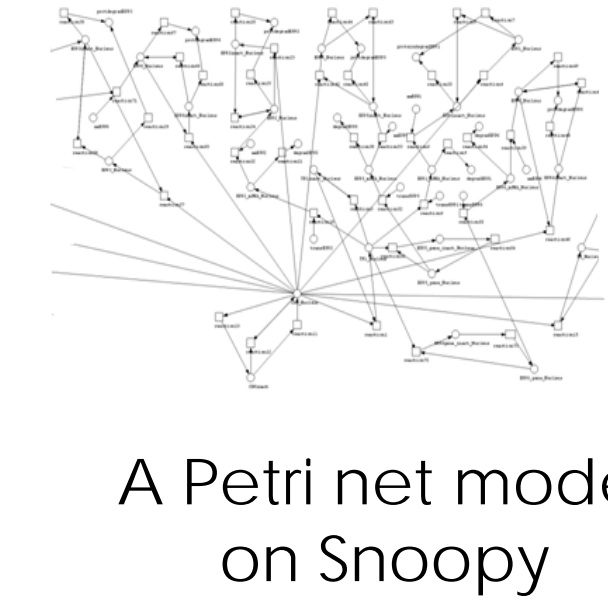
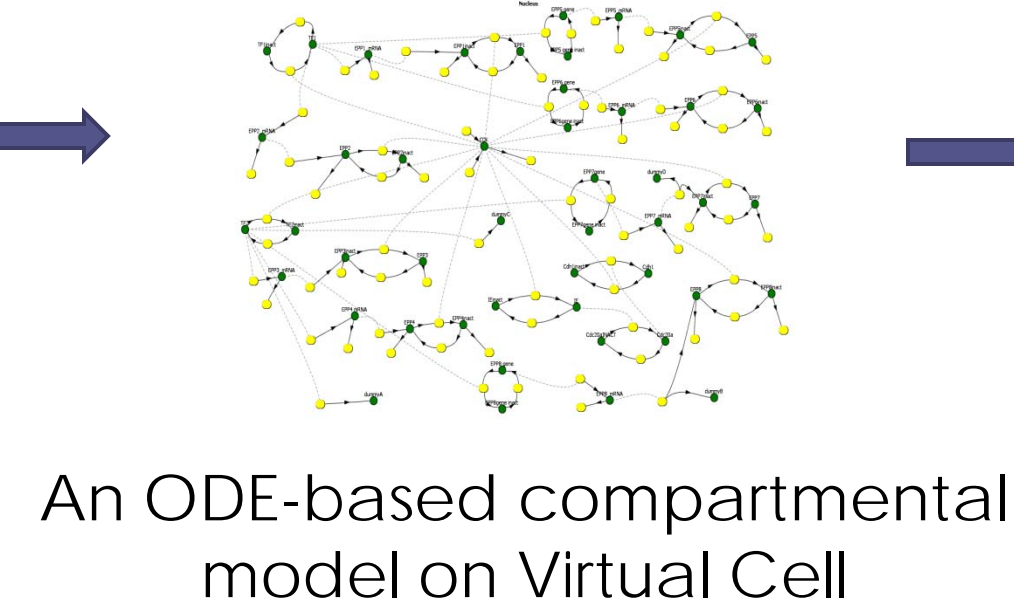
Long term potentiation (LTP) can elicit biological effects in processes such as neoplastic transformation and learning and memory. In the hippocampus, calcium ions can activate CAMKII, increasing synaptic responses. CAMKII can still have sustained activity even after the  $Ca^{2+}$  concentration drops



## Methods

Input parameters, reactions and species

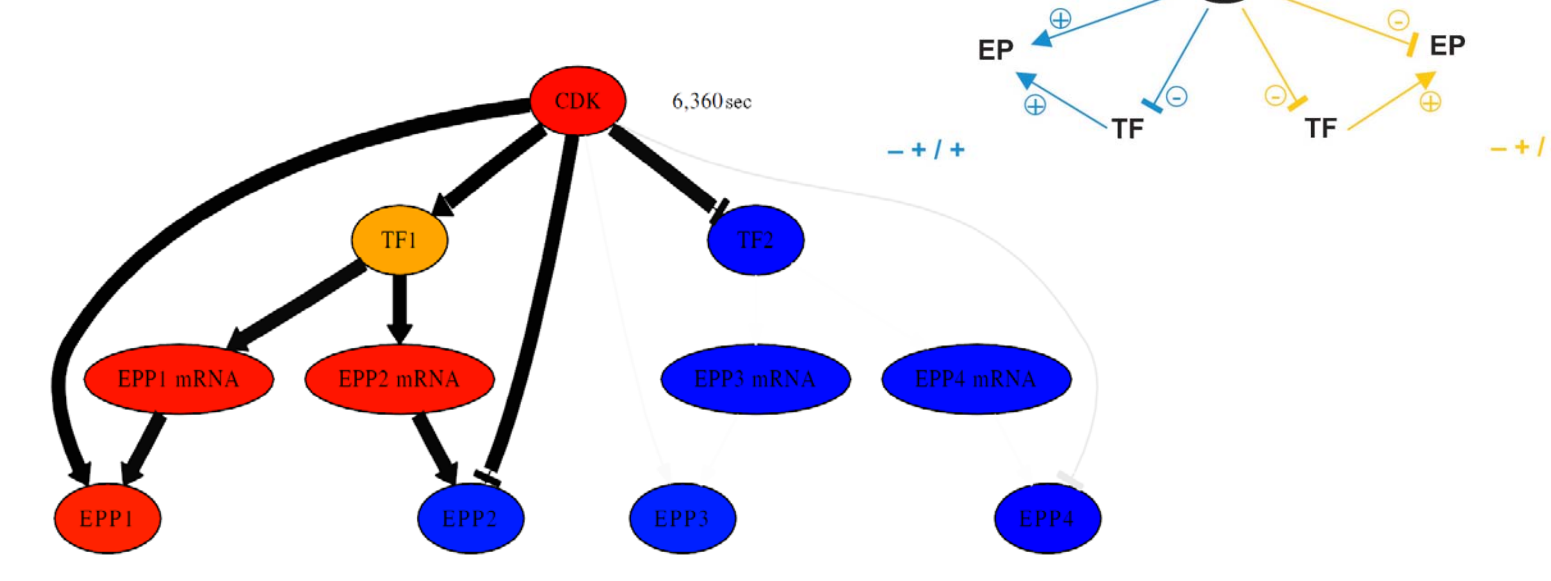
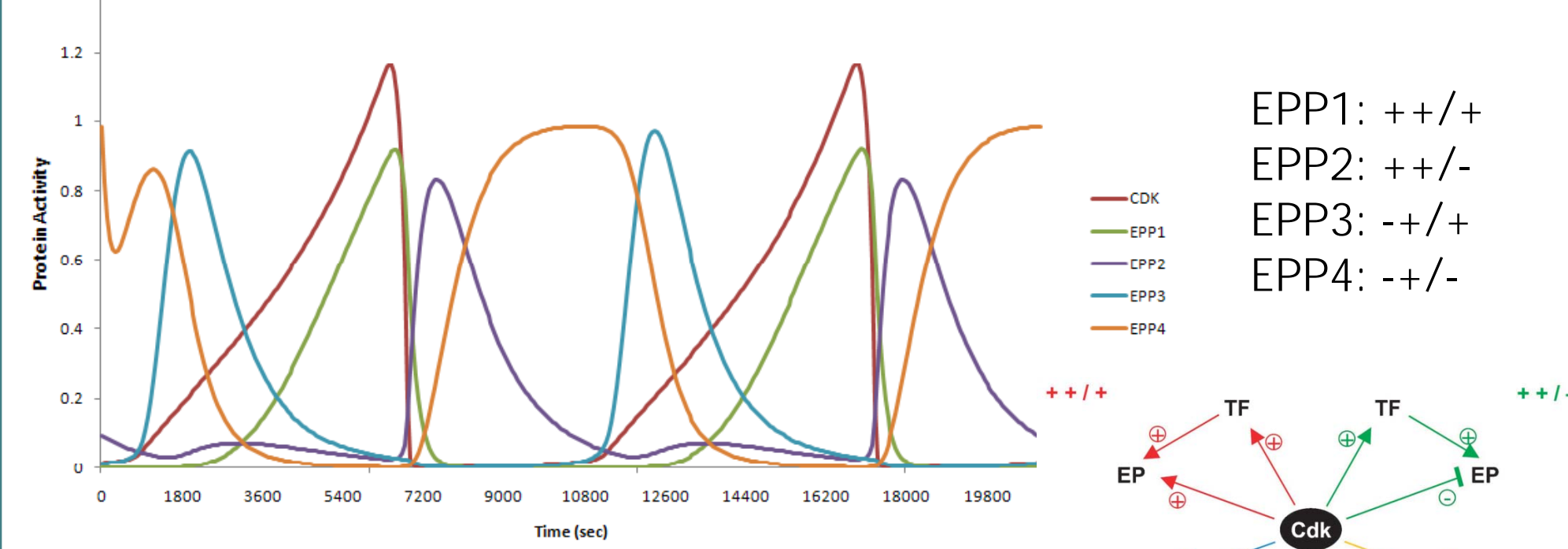
From published model



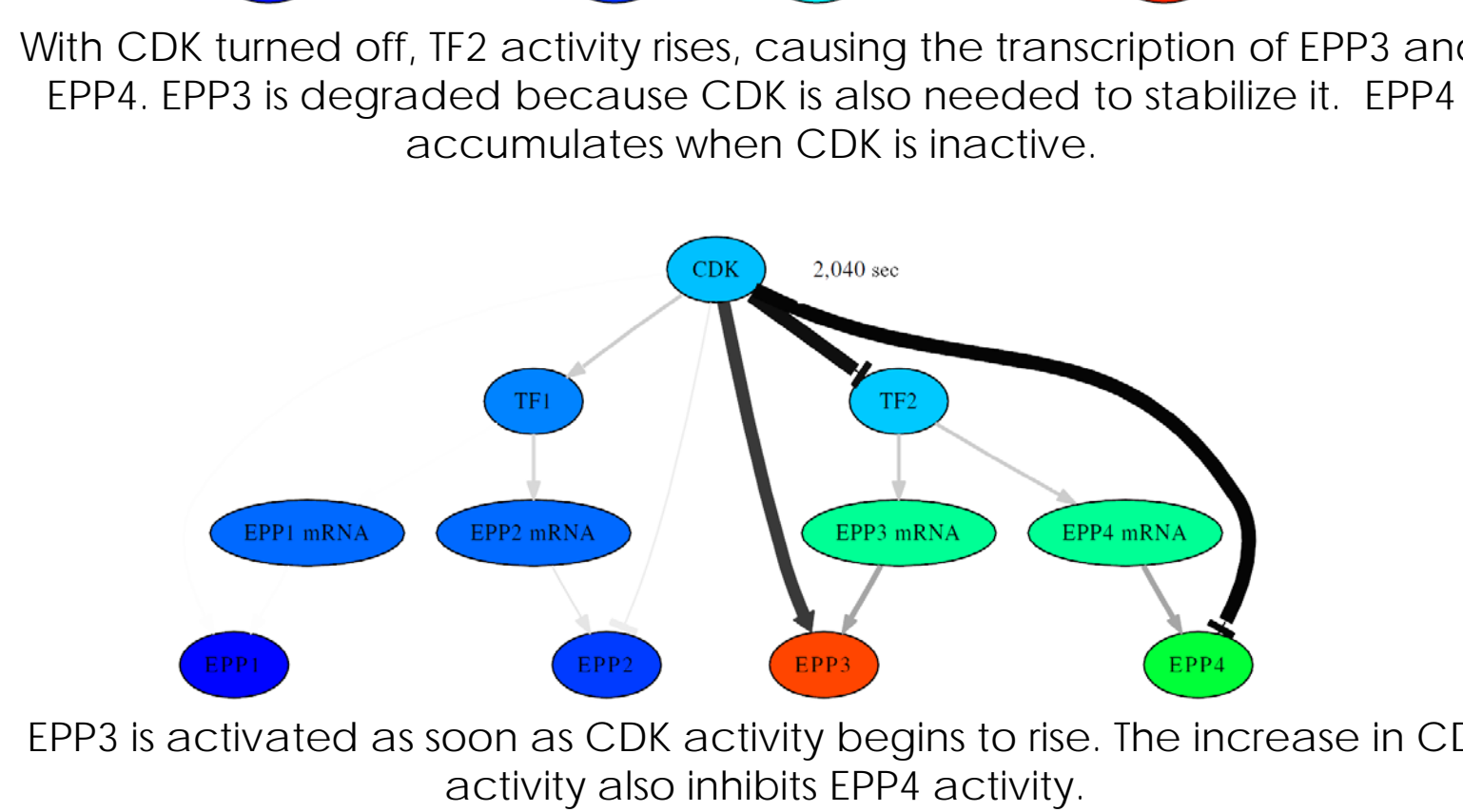
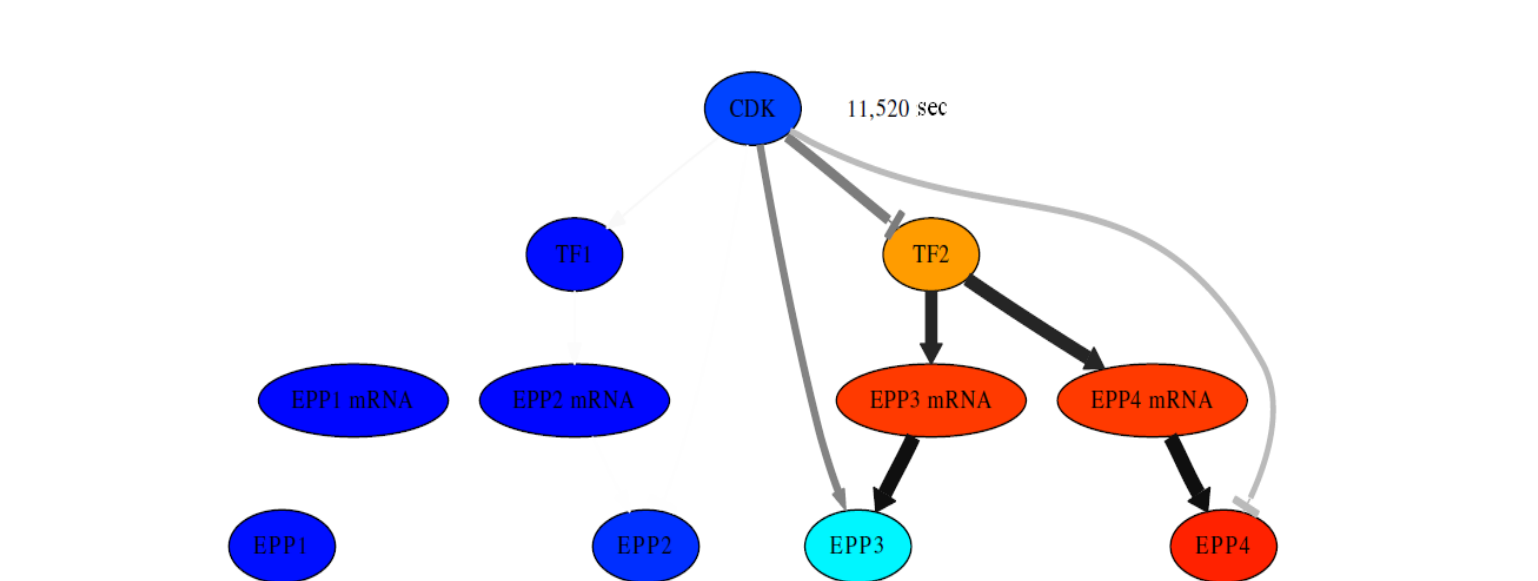
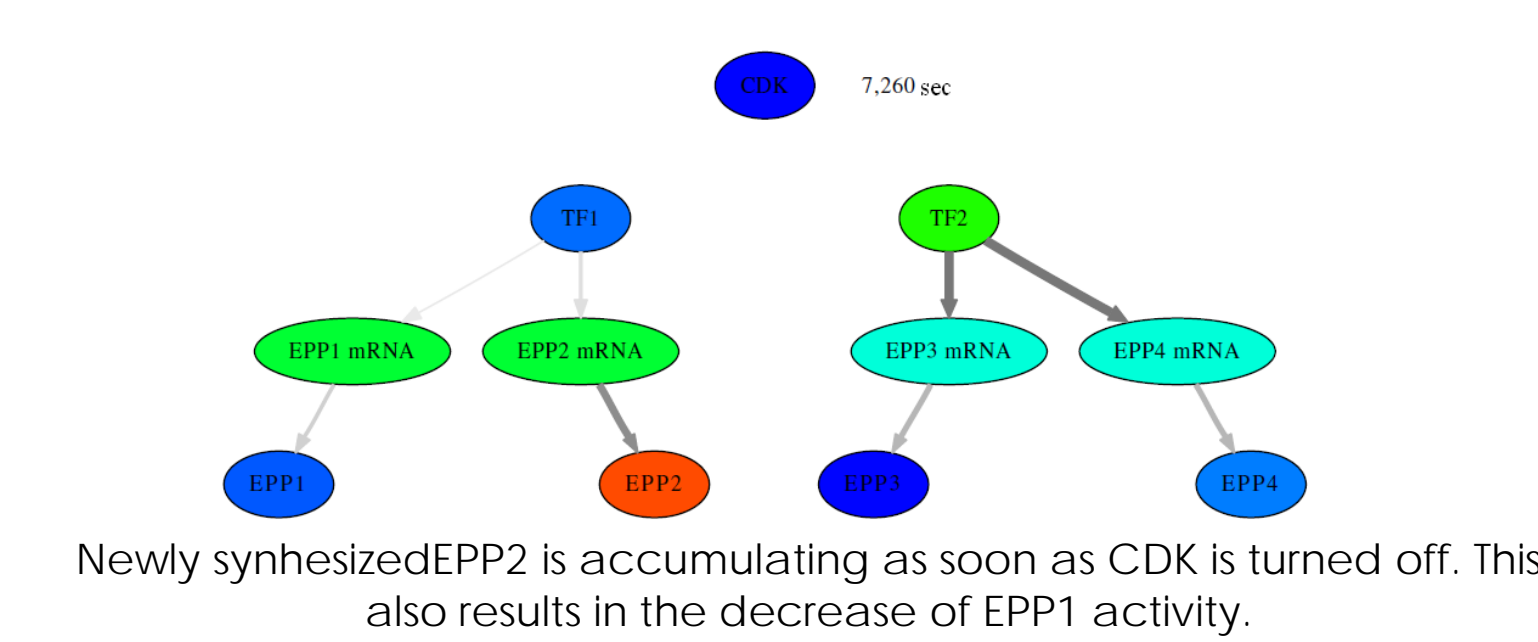
## Results

### Cell Cycle

The function of FFL depends on signs + for activation or - for inhibition.  
 1. indicates the effect of Cdk phosphorylation on the activity of TF.  
 2. indicates the effect of the active form of TF on gene expression.  
 3. indicates the effect of direct phosphorylation of EP by CDK.

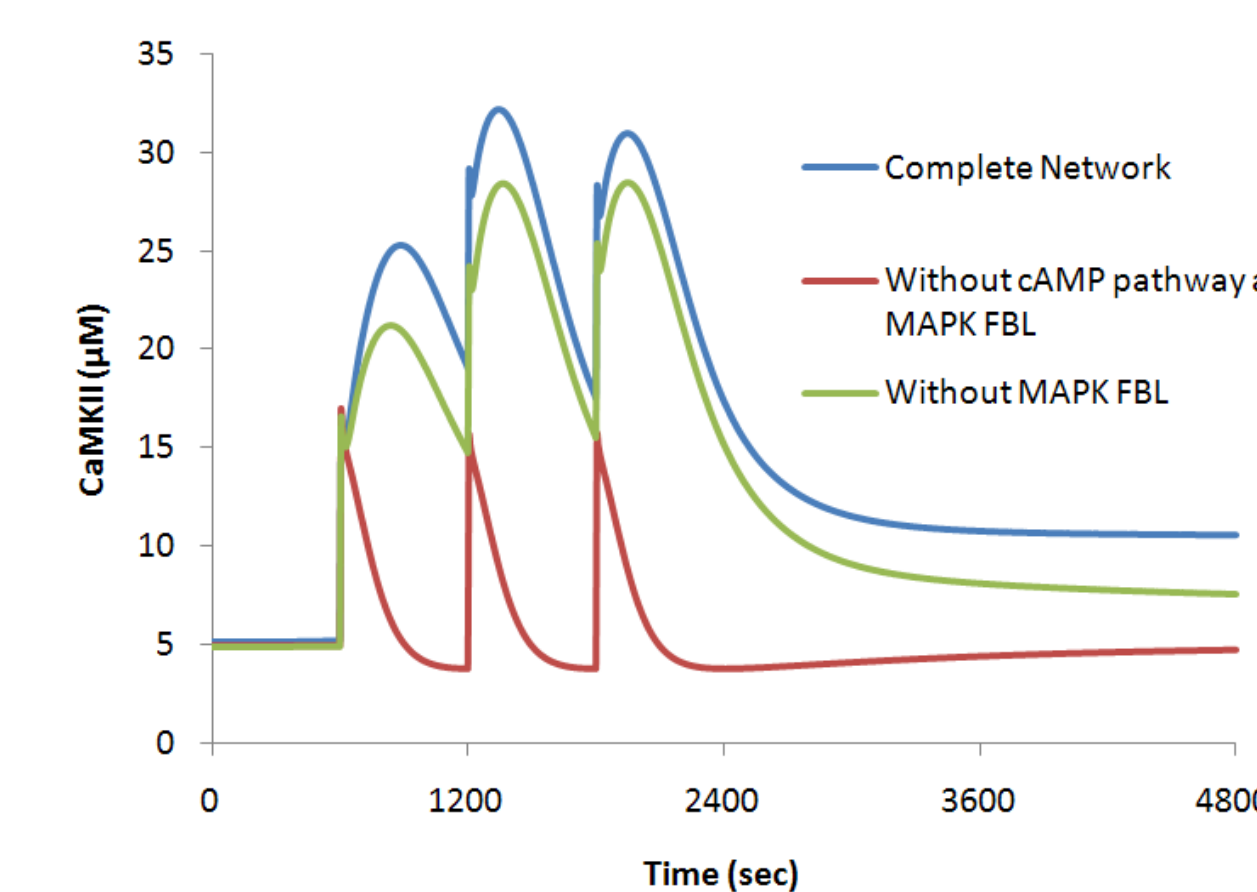


EPP1 reaches maximum activity with both CDK directly activating it and EPP1 mRNA synthesizing it. EPP2 mRNA is active but no EPP2 can become active with CDK inhibiting EPP2.

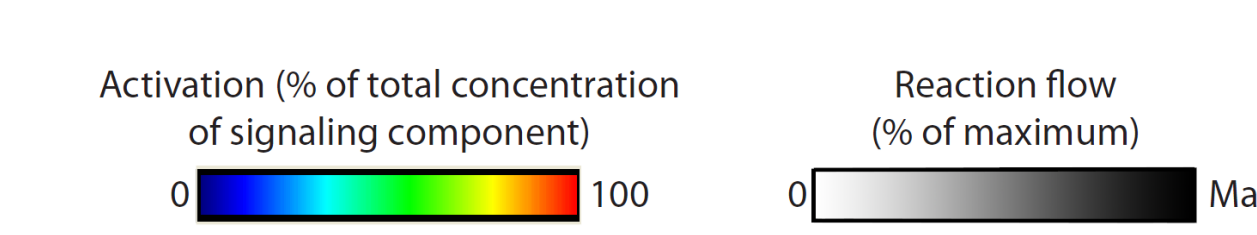
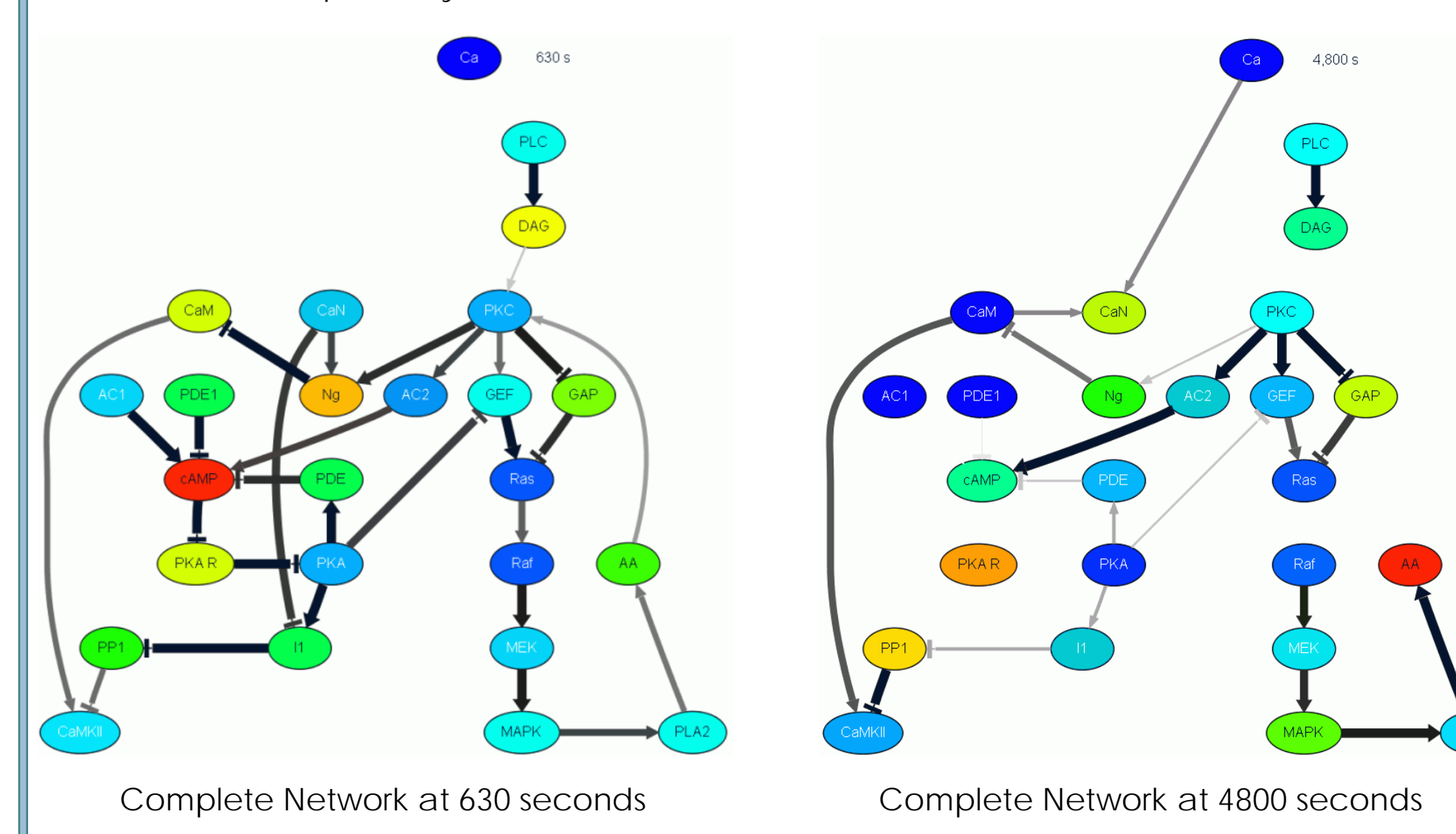
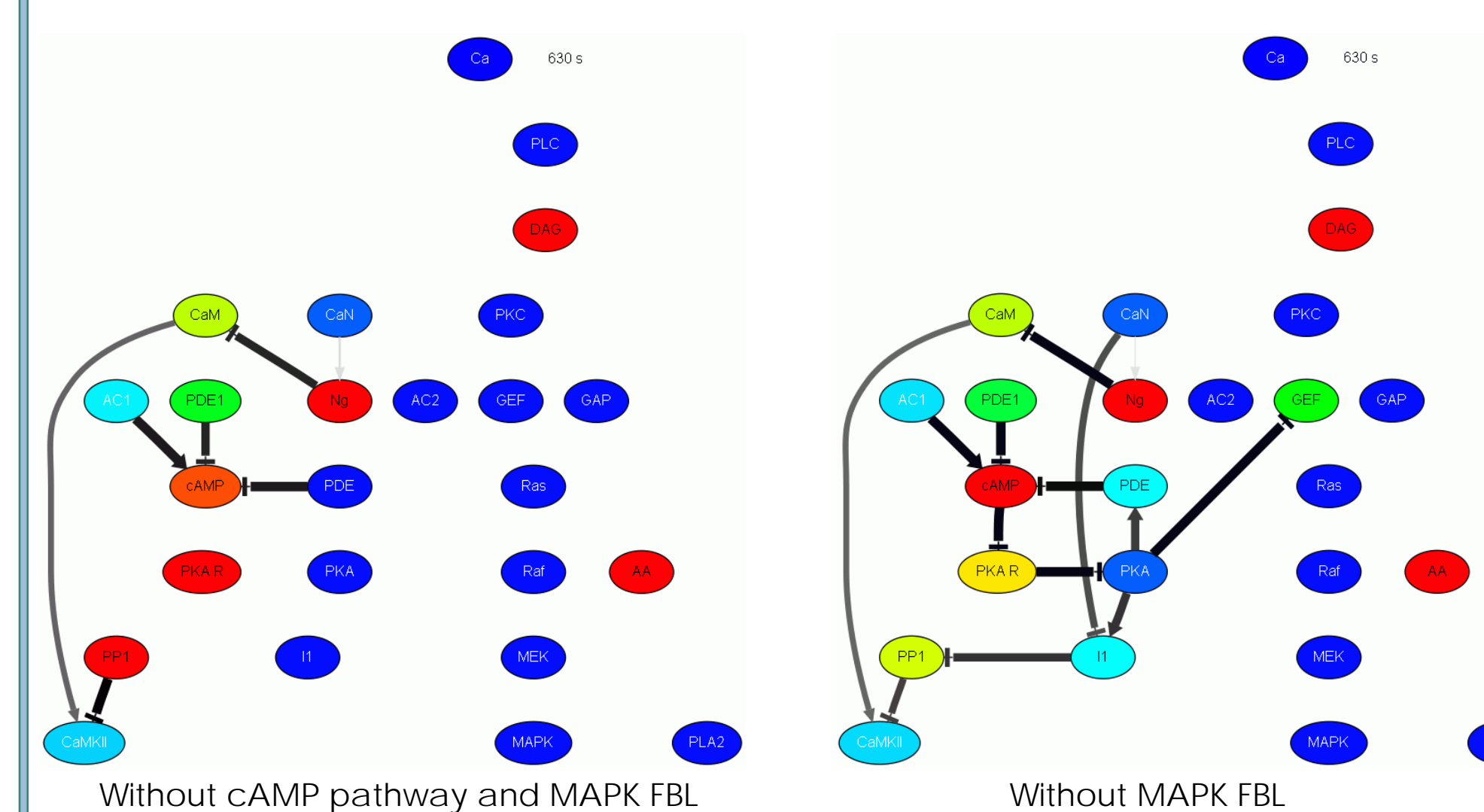


### LTP of CaMKII

cAMP Pathway:  $Ca^{2+}$  ions elevate cAMP through calmodulin and adenylyl cyclases 1/8. This causes an increase of PKA activity which leads, in our network, to the inhibition of PP1. Inactive PP1 is then unable to deactivate CAMKII. PKC-MAPK FBL: Glutamate induced  $Ca^{2+}$  ions activate CAMKII, PKC, PKA, and MAPK. In this network of four protein kinase pathways, a positive feedback loop is created by the connection between the PKC and MAPK pathways.

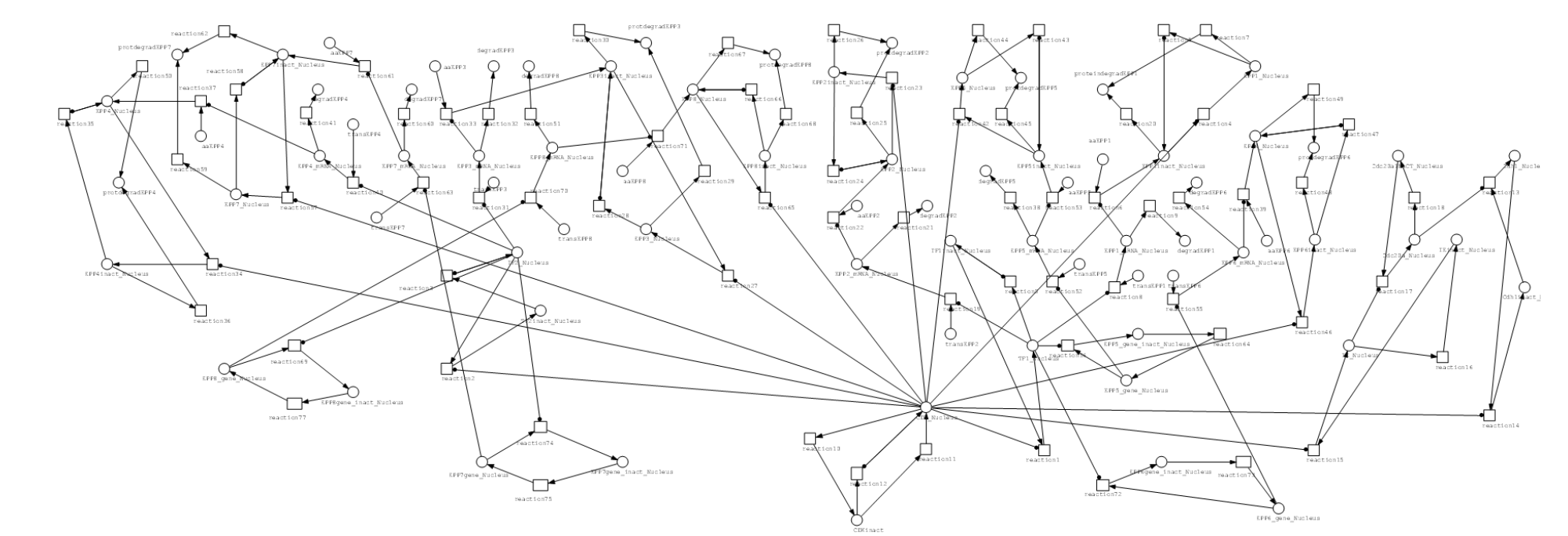


With only a direct activation by calmodulin, CAMKII reaches low activity and returns to basal state. With the cAMP pathway forming the second path of a FFM, the CAMKII response is amplified but returns to basal levels. The addition of the MAPK FBL nested within the FFM produces a sustained CAMKII active state.



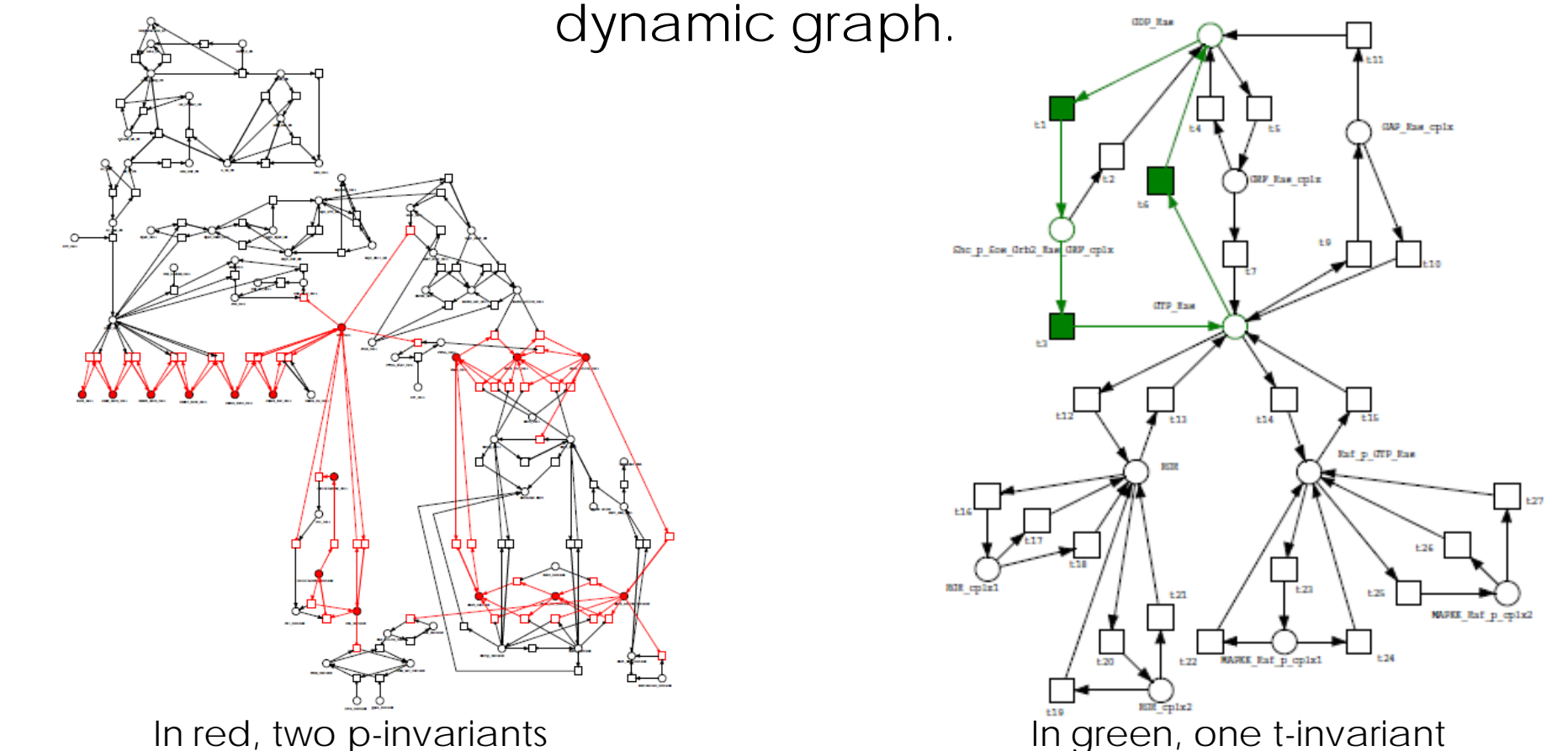
## From Petri Net to Dynamic Graph

An ODE-based compartmental model is converted into a Petri net model using Snoopy which is then analyzed for conservative invariants or p-invariants and t-invariants using Charlie.



The Petri Net model of the cell cycle

P-invariants contain a property which states that the markings of such a set of places form a linear combination that is always constant no matter what the state of the system. Each p-invariant is a node in the dynamic graph. T-invariants reveal the signaling components of the model. They are represented as edges going from one node to the next in the dynamic graph.



In red, two p-invariants

In green, one t-invariant

Each node and edge in the dynamic graph is assigned a rule that converts simulation data into colors, creating an animated representation of a signaling network. The color of the nodes and edges represents the level of activity of a molecular species or of a reaction flow at a specific simulation time step.

## Conclusion

- The dynamic graphs correspond to the simulations for both the cell cycle and the LTP model, showing that dynamic graphs can be used to efficiently analyze the interrelated dynamics of motifs. This is key to understanding the properties of complex interactions in biological systems. The activity state of motifs is more informative than the activity states of individual signaling molecules.
- The effect of the + and - signs in the regulatory steps of FFLs determine the activation of different proteins at different time steps in the cell cycle.
- The interaction of the cAMP pathway and the PKC-MAPK positive feedback loop causes sustained increase in CAMKII activity.

## Acknowledgements

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## References

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