

Using large scale, multi-cellular pathway modeling to understand cellular differentiation

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Abstract

Genetic expression and control pathways can be successfully modeled as electrical circuits. Given the vast quantity of genomic data, very large and complex genetic circuits can be constructed. To tackle such problems, the massively-parallel, electronic circuit simulator, XyceTM [10], is being adapted to address biological problems. Unique to this bio-circuit simulator is the ability to simulate not just one or a set of genetic circuits in a cell, but many cells and their internal circuits interacting through a common environment. Additionally, the circuit simulator Xyce can couple to the optimization and uncertainty analysis framework Dakota [2] allowing one to find viable parameter spaces for normal cell functionality and required parameter ranges for unknown or difficult to measure biological constants.

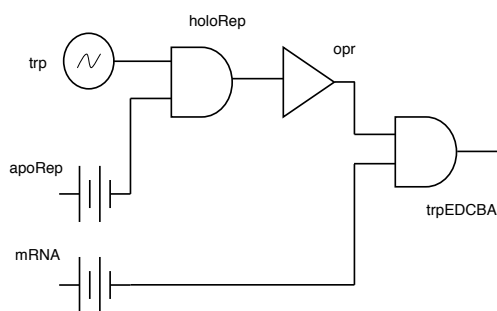
Currently, electric circuit analogs for common biological and chemical machinery have been created. Using such analogs, one can construct expression, regulation and reaction networks. Individual species can be connected to other networks or cells via non-diffusive or diffusive channels (i.e. regions where species diffusion limits mass transport). Within any cell, a hierarchy of networks may exist operating at different time-scales to represent different aspects of cellular processes.

Though under development, this simulator can model interesting biological and chemical systems. Here, we investigate Dassow's *Drosophila sp.* cellular differentiation network's stability as a function of initial conditions. [3] For these computations, a collection of 100 cells connected through a diffusive environment are simulated within the circuit modeler Xyce while the optimization engine, Dakota, controls cellular pathway parameters. Our findings agree with Dassow's that the overall network is fairly robust. However, when one includes smooth initial conditions the ranges of acceptable parameter values shrink as well as the complexity of the final solution.

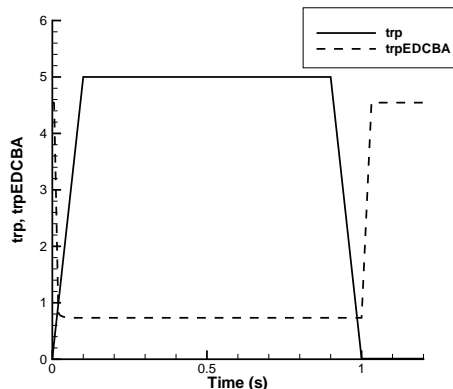
Introduction

Expression of a genetic code defines characteristics of a given organism. As an organism grows and adapts to its local environment specific elements of its genetic code are expressed while other elements are suppressed. Complex control mechanisms exist to regulate the expression of genes during the life of a cell. [5]–[7]

To fully appreciate how a genetic repository or genome translates into a functioning cell, one must understand the control mechanisms of genetic expression. Genetic products of a given gene can promote or suppress the further production of that gene creating a simple feedback loop. [5]–[7] Similarly, genetic products from other genes can regulate the production of a given gene creating complex feedback loops or expression cascades. Feedback loops and cascades are not limited to a single cell, but can span an entire cell culture or cellular generation influencing differentiation and development. [6]



A)



B)

Figure 1: A) A tryptophan regulated switch. An oscillating input level of tryptophan, trp , controls the production of the gene products $trpEDCBA$. B) The tryptophan switch in action. Only when tryptophan, trp , levels drop are the gene products, $trpEDCBA$, available.

As an abstraction to better understand genetic expression and control, genetic material and its associated control mechanisms can be viewed as a genetic switch. [1], [5]–[8] Such a switch can be modeled as an electrical circuit where a signal (the transcript from a section of DNA) is generated and altered as it interacts with other components during its propagation. This analogy is far from perfect as there are significant differences in the switching speed and signal to noise ratio of a genetic circuit versus an electric circuit. However, this analogy allows one to consider very complicated, dynamic control circuits while investigating expression stability and population dynamics. [6]

Framework

A biological or chemical simulation working within an electrical circuit context requires a translation framework to convert from the former domain to the latter. In electronics, a fundamental quantity is charge, denoted q . In the biological domain, one is often concerned with concentration of a given chemical species. Given a control volume, such as the volume of a cell, concentrations can be converted to mass. As a basis for a biological to electrical problem conversion, this work will equate mass of a given chemical species with charge. Each pathway or *wire* in a circuit will carry a different chemical species and the charge on that pathway will denote the mass of that chemical species present in the simulation.

Continuing this analogy, electrical current which is the timed rate of change of charge is equivalent to the rate of mass change, i.e. how quickly a compound is used or created by the system. Voltage corresponds to chemical concentration, while Kirchoff's Voltage Law enforces stoichiometric balance on chemical reactions and Kirchoff's Current Law enforces conservation of mass within the system.

Genetic Switch Application

As an example of a biologically inspired circuit, figure 1A demonstrates a genetic switch in an *E. coli* tryptophan regulation circuit. Focusing primarily on the switching, this problem is posed as a digital circuit. Thus, specific reaction rates and binding constants are ignored so that only tryptophan induced regulation is seen. Casting the problem this way allows one to study cases where little or no kinetic, stoichiometric or diffusion data are available and one wishes to test hypothetical control systems.

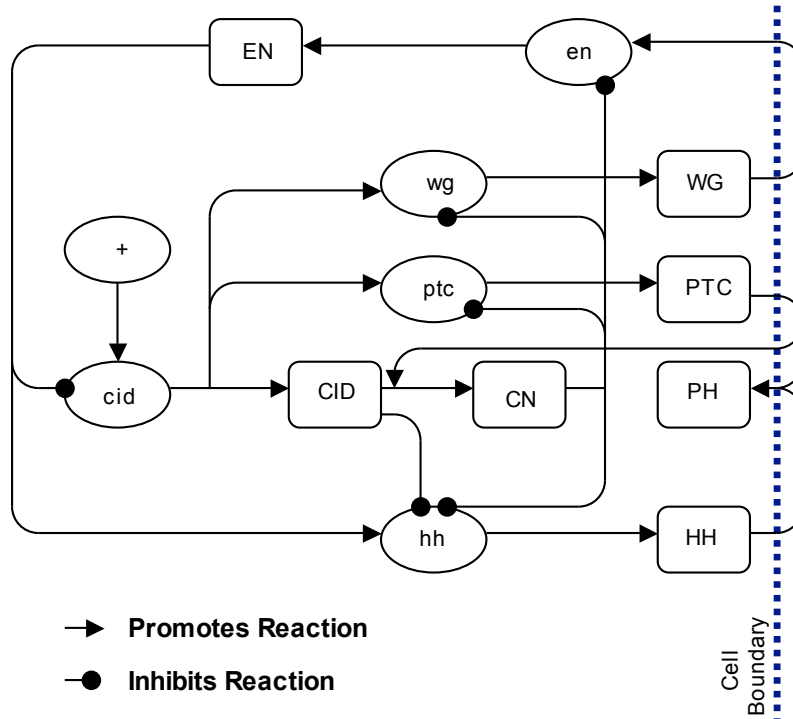


Figure 2: Developmental control circuit derived by Dassow [3]. Lower case letters are mRNA (gene products) while upper case letters denote proteins. Arrows indicate places where a compound promotes the production of another compound while filled circles denote places where a compound represses the production of another species.

In figure 1A, a repressor, apoRep, and mRNA are preset at constant levels and depicted as constant voltage sources. Tryptophan, trp, concentration oscillates and it is implemented as a time dependent source. If trp and apoRep are present then an activated repressor holoRep is formed as indicated by the AND gate. The presence of the activated repressor deactivates the operator, opr via a NOT gate. Without opr, production of the tryptophan controlled gene products, trpEDCBA, is shut down. Figure 1B graphically shows the genetic reacting to changes in the applied tryptophan level. While this example is very simple, it demonstrates that biological control concepts can be mapped into a circuit model. More importantly, this simple switch is easily embedded in a larger circuit allowing one to model complex systems from simple constituents.

Modeling Developmental, Cyclical and Feedback Pathways

Development of a system from one state to another in a controlled manor usually involves feedback to assert such control. From purely chemical systems such as the Belousov-Zhabotinskii (BZ) reaction network [9] to multi-cellular networks such as *Drosophila sp.* differentiation [3] use feedback to control the system's development.

During development to of the fruit fly embryo, *Drosophila sp.*, a series of dark bands develop along the major axis of the growing larva—a graphical indicator of the underlying cellular differentiation in progress. Figure 2 presents the control network responsible for cellular differentiation in *Drosophila sp.* [3]. Though complex, this network typically bifurcates into one of two states. If a cell is producing

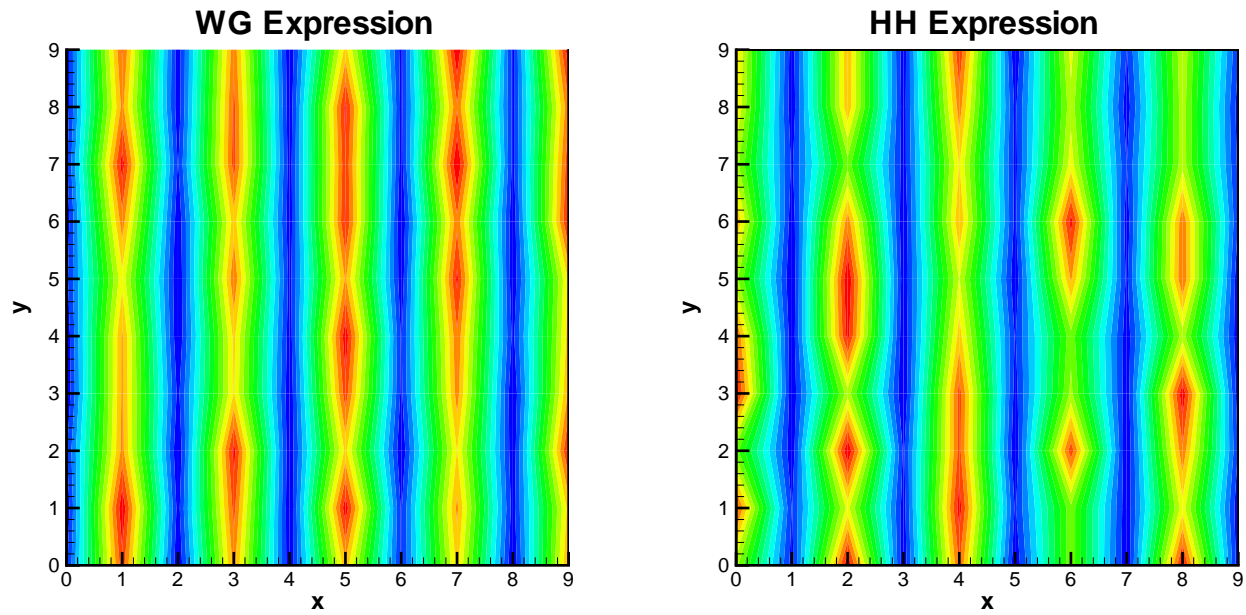


Figure 3: A 10 by 10 grid of cells starting with an initial noisy, oscillatory level of WG differentiates into WG producing and HH producing populations. The plot on the left depicts 5 layers of WG producing cells while the right contour plot depicts 5 layers of HH producing cells at the same timepoint. Initially the system was started with 10% rms. random noise in WG superimposed over the initial conditions.

the gene product wg then the protein WG will likely be produced as well. The WG protein is exported into the cellular environment and picked up by neighboring cells where it can promote the expression of the gene product en . The en gene product represses the production of wg and puts the cell into a different state from a cell producing WG, specifically into a state where it is producing and expressing HH. Thus, cells will typically be producing either WG or HH with a small percentage of cells producing both of these proteins as the switch from expressing one gene to another.

Actual simulations of the *Drosophila sp.* network were carried out as follows. The network was converted to an electrical circuit using analogs for chemical reactions, material storage, promotion, repression, degradation and diffusion. These analogs treat electrical charge, a conserved quantity in electrical circuit simulators as a mass conserving mass within the system. Once the circuit was created, a 10 by 10 grid of cells embedded within a diffusion limited environment was created, again as a circuit. Fundamental constants like reaction rates, enzymatic turnover rates and diffusion coefficients were parameterized within this circuit. Such parameterization allows the optimization framework to alter parameters between simulation runs to explore the phase space for this system.

Figure 3 depicts concentration contour plots of the species WG and HH. Initially, the system was started with zero concentration of the exported species, PH, PTC and HH and an oscillatory level of WG. This initial oscillatory state represents the initial bias that anterior-posterior/dorso-ventral patterning hierarchies initiate in the developing embryo. [4] Additionally, a 10% rms. random noise was added to the WG initial conditions to simulate disturbances of the system from an ideal starting state. Such noise was also parameterized in the circuit and varied to gauge system robustness. Physically, the striations in concentration shown in figure 3 represent layers of cells becoming WG producing or HH producing over time an example of cellular differentiation.

With expression, enzymatic turn-over, reaction and diffusion rates and noise levels all parame-

terized, a design of experiments approach was used to understand how this collection of state variables affects the resulting system. Using the Dakota optimization framework, the problem parameter space was explored using latin-hypercube sampling. As expected, system robustness decreases as more initial noise was added; full results will be presented in our poster.

Conclusion

Though still in development, this biological circuit simulator has the potential to handle large and complex problems. Depending on the type of data available, one can cast problems as digital or analogs circuits and easily simulate many replica of a single circuit interacting with a collection of other circuits. Through the coupling to an optimization framework, one can explore the dynamics of multiple cellular networks or of entire cell cultures elucidating governing parameters as well.

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