Computational techniques in mathematical modelling of biological switches

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Abstract: Mathematical models of biological switches have been proposed as a means to study the mechanism of decision making in biological systems. These conceptual models are abstract representations of the key components involved in the crucial cell fate decision underlying the biological system. In this paper, the methods of phase plane analysis and bifurcation analysis are explored and demonstrated using an example from the literature, namely the synthetic genetic circuit proposed by Gardner et al. (2000) which involved two negative loops (from two mutually inhibiting genes). Figure 1 shows a schematic diagram of the synthetic genetic circuit constructed by Gardner et al. (2000). Particularly, a saddle-node bifurcation is used as a signal response curve to capture the bistability of the system. The notion of bistability is obscure to most novice researchers or biologists because it is difficult to understand the existence of two stable steady states and how to flip from one stable steady state to another and vice versa. Thus, the main purpose of this paper is to unlock the computational techniques (bifurcation analysis implemented in a software tool called XPPAUT) in mathematical modelling of bistability through a simple example from Gardner et al. (2000). In addition, time course simulations are provided to illustrate: 1) the notion of bistability where the existence of two stable steady states and we demonstrated that for two different initial conditions one of the genes is 'ON' and the other gene is 'OFF'; 2) hysteresis behaviour where the saddle-node bifurcation points as two critical points in which to turn 'ON' one gene happens at a larger parameter value than to turn 'OFF' this gene (at a lower parameter value). The hysteresis behaviour is important for irreversible decision made by cell to commit to turn 'ON'. In conclusion, the understanding of the computational techniques in modelling biological switch is important for elucidating genetic switch that has potential for gene therapy and can provide explanation for experimental findings of bistable systems.
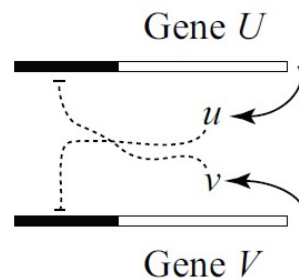
Figure 1. The construction of a genetic toggle switch by Gardner et al. (2000), who used bacterial plasmids to engineer this circuit in a living cell. Here the two genes, U, V produce products *u*, *v*, respectively, each of which inhibits the opposite gene's activity. (Black areas represent the promoter region of the genes.) Copyright © 2013 Society for Industrial and Applied Mathematics. Reprinted with permission. All rights reserved.

Keywords: *Biological switch, mathematical model, saddle-node bifurcation, bistability*
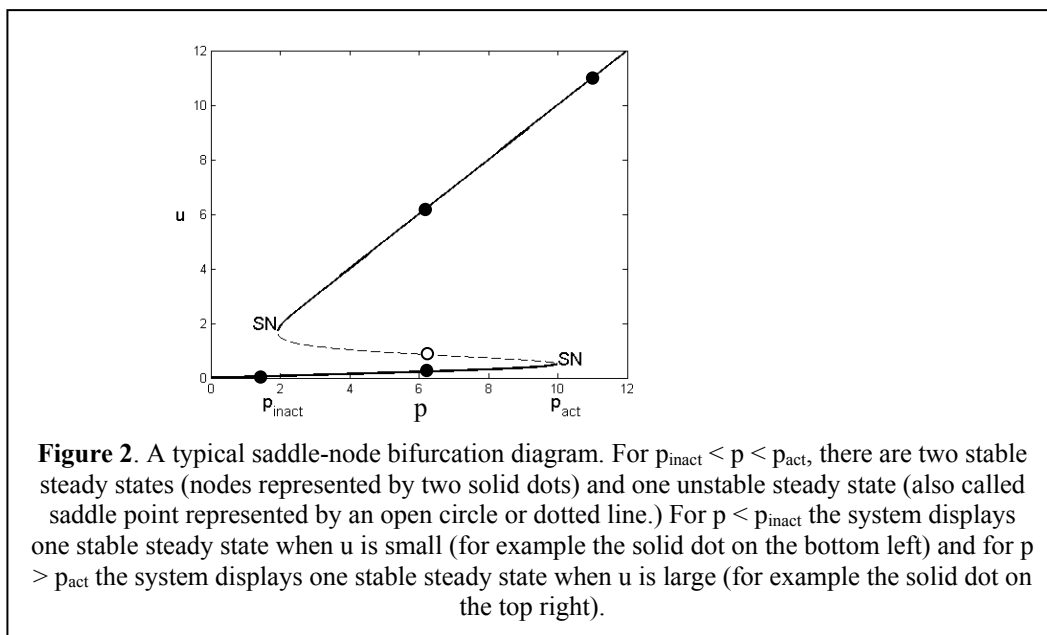
1.   INTRODUCTION

The objectives of mathematical modelling of biological systems are to formulate conceptual models of the molecular mechanism underlying the biological system and to provide a means to study the mechanism that controls the dynamics of the system. There are a few excellent review papers, for examples, Tyson et al. (2001), Goldbeter (2002) and a book (Segel & Edelstein-Keshet, 2013), that serve as an introductory to the study of conceptual models. These review articles and the book proposed methods that closely link experimental observations of protein-level oscillations and bistable hysteresis behaviours with bifurcation diagrams. The concept of bifurcation point comes from the dynamical system of a model (biochemical reaction model) where stability of fixed point changes when a parameter is varied from stable to unstable or stable fixed point is created or destroyed. The bifurcation point indicates interesting dynamics of the system that correspond to behaviour of protein concentrations, for example the system reach a stable fixed point or starts oscillating. Particularly, the computational technique applied bifurcation theory in generation of bifurcation diagram as a signal response curve — to explain the changes in a specific parameter (reaction rate representing signal) in the model and the response (the observation of a certain protein concentration level that indicates certain cell behaviour or physiology) (Tyson et al., 2001). In general, the use of Hopf bifurcation is used to explain oscillatory behaviours and the use of saddle-node bifurcation is used to explain biological switch. However, the method of saddle-node bifurcation that is used as a means to study biological switch is often obscure to novice researchers and biologists. Thus, this paper aims to review (and illustrate) the concepts of bistability and hysteresis behaviour through a simple example from the synthetic genetic circuit proposed by Gardner et al. (2000) which contains two mutually inhibiting genes.

The overall goal of this paper is to provide a detailed discussion and hands-on description on mathematical modelling techniques to novice researchers or biologists — readers are assume to have some background knowledge in dynamical system theory such as the textbook in (Strogatz, 1994) — who are interested in the modelling of biological switches.

2.   THE CONCEPT OF BISTABILITY CAPTURED BY SADDLE-NODE BIFURCATION

In this section, we are going to introduce the concept of bistability that is captured by a saddle-node bifurcation, which is a specific type of bifurcation diagram. The application of bifurcation diagram to the analysis of cell physiology is based on the correlation of the qualitative changes in the attractors and repellers of a vector field that correspond to the qualitative changes in the state of cell physiology (Tyson et al., 2001). Attractors are stable steady states and repellers are unstable steady states. For example, a saddle-node bifurcation diagram is used to describe a bistable system that exhibits bistability and hysteresis behaviour (hysteresis behaviour will be discussed in Section 3.4). A typical example of saddle-node bifurcation diagram with two saddle-node points (the two marked as SN) is shown in Figure 2 (Tyson, 2011). It shows the signal-response curve: the changes in the signal (the x-axis represented by a parameter, p) with the corresponding response in a gene expression (the y-axis represented by a variable, u).

Figure 2. A typical saddle-node bifurcation diagram. For $p_{inact} < p < p_{act}$, there are two stable steady states (nodes represented by two solid dots) and one unstable steady state (also called saddle point represented by an open circle or dotted line.) For $p < p_{inact}$ the system displays one stable steady state when u is small (for example the solid dot on the bottom left) and for $p > p_{act}$ the system displays one stable steady state when u is large (for example the solid dot on the top right).

Let us look into the detail of the notion of bistability. According to Figure 2, the saddle-node (SN) bifurcation diagram illustrates qualitative changes from one stable steady state to the behaviour of two stable steady states or bistability. For the parameter values between the two thresholds, $p_{inact} < p < p_{act}$, there exist two stable steady states and one unstable steady state. The bistability of a system can be visualized in a phase plane analysis (Figure 3 (b)). Phase plane is a plane of two variables showing vector fields and the flow of the trajectories for any combination of initial points (or initial conditions) in this plane (an example of flow in a system is shown in Figure 4). A nullcline is the solution (as a curve drawn in the phase plane) when the derivative of a model variables in the system of differential equations is set to zero. Figure 3 (b) shows two nullclines (represented by green and brown lines) and the intersection points from these nullclines are the steady state points of the system.

For $p_{inact} < p < p_{act}$, there are two stable steady states (nodes represented by two black dots in Figure 3 (b)), which are attractors and the indication of the existence of bistability. The system can be attracted to either one of the stable steady states, which depends on the state of the system or initial conditions. The unstable steady state (also called saddle point represented by an open circle in Figure 3 (b)) is a repeller. At the thresholds, $p = p_{inact}$ and $p = p_{act}$, one of the nodes and the saddle point coalesce and disappear (Tyson, 2011). This is the reason why it is called "saddle-node bifurcation". For $p < p_{inact}$, the system displays one stable steady state when u is small (Figure 3 (a)) and for $p > p_{act}$, the system displays one stable steady state when u is large (Figure 3 (c)), which corresponds to the cell physiology of a gene u getting turned off or turned on, respectively. Another feature of saddle-node bifurcation is the hysteresis behaviour: the signal required to turn on the gene is p_{act} which is different from the signal to turn off the gene at p_{inact}, and p_{inact} is much smaller than p_{act} (hysteresis behaviour will be discussed in more details in Section 3.4).

a) $p < p_{inact}$ b) $p_{inact} < p < p_{act}$ c) $p > p_{act}$
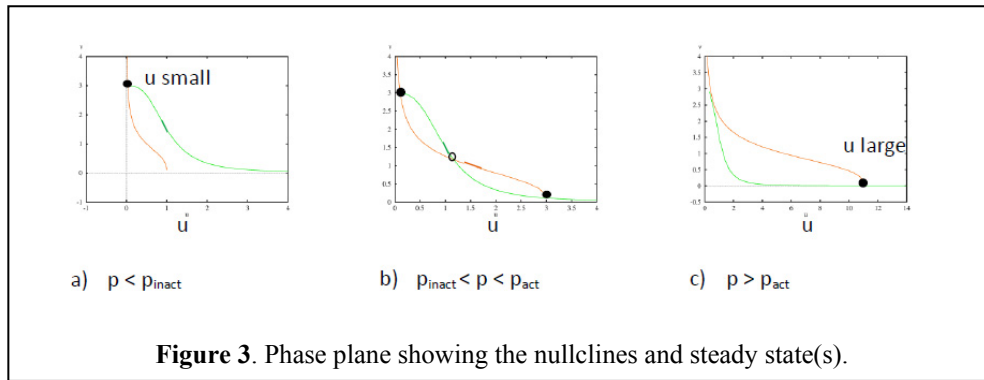
Figure 3. Phase plane showing the nullclines and steady state(s).

3.   BISTABILITY IS ILLUSTRATED WITH A SIMPLE EXAMPLE OF SYNTHETIC GENETIC CIRCUIT FROM GARDNER ET AL. (2000)

The synthetic genetic switch proposed by Gardner et al. (2000) is a classic example from synthetic biology, which was constructed from two mutually inhibiting genes (see Figure 1). In other words, one of the genes U encodes protein *u* that inhibits the transcription of another gene V, which encodes protein *v* that inhibits the transcription of gene U. The understanding of this synthetic biochemical switch may have biomedical implications in gene therapy in future as reported by Gardner et al. (2000). They proposed a mathematical model for the genetic switch to gain a deeper understanding through predictions from the model that guided their experiments, and it is given by two differential equations below:

$$\mathrm{d}u/\mathrm{d}t = \alpha_1/(1+v^n) - u \tag{1}$$

$$\mathrm{d}v/\mathrm{d}t = \alpha_2/(1+u^m) - v \tag{2}$$

Here, *u* and *v* are the concentrations of proteins from gene U and gene V, respectively. In Equation (1), the first term describes inhibition of gene U by protein *v* and the second term describes the degradation of protein *u* with a constant rate. In Equation (2), the first term describes inhibition of gene V by protein *u* and the second term describes the degradation of protein *v* with a constant rate.

Table 1 shows a set of model parameters chosen from Segel & Edelstein-Keshet (2013) as an example for illustrating the phase plane, and the bifurcation analysis. The parameter values are used because it can produce saddle-node bifurcation; for example, a bifurcation diagram of the variable *u* against α_1, where α_1 is chosen as a bifurcation parameter by varying α_1 and keep track of steady state (and stability) changes in *u* (as will be discussed in Section 3.3).

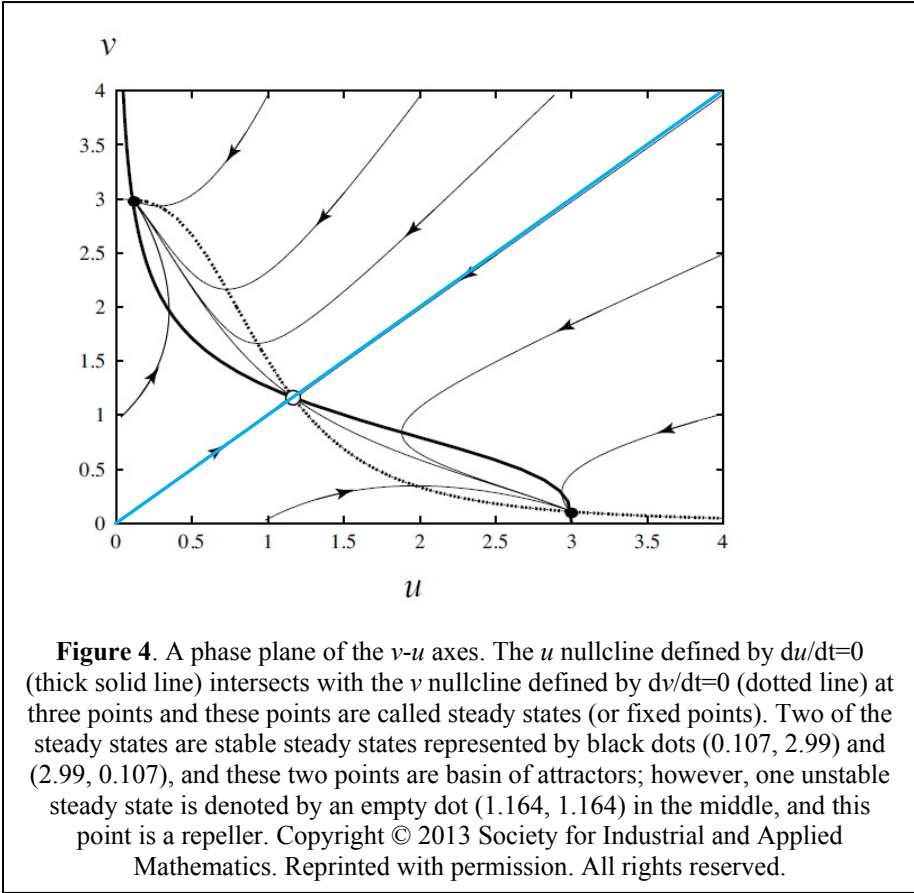**Table 1**. The parameters for the differential equations.

No.	Parameter	value
1.	α_1	3
2.	α_2	3
3.	n	3
4.	m	3

The software tool XPPAUT is used to plot the phase plane and bifurcation diagram. It can be downloaded freely from http://www.math.pitt.edu/~bard/xpp/xpp.html. It is a tool for studying dynamical systems and performing computer simulations (time course simulation, phase plane analysis and bifurcation diagram). In the following sections, we will discuss the phase plane analysis, bifurcation diagram and time course simulations for illustrating the notion of bistability and hysteresis behaviour.

3.1. Phase plane analysis

Assuming that you have downloaded the XPPAUT software and following the instructions for setting up the system you have been able to run XPPAUT in your computer. There is a tutorial for beginner on how to start with creating and running ODE file, please refer to the download webpage mentioned earlier. The ODE file is a file ended with .ode, where you can use a text editor such as notepad to create and save the ODE file. For the Gardner et al.'s model, a phase plane can be plotted for the two nullclines.

The phase plane for Gardner et al.'s model is shown in Figure 4. The differential equations above with the chosen parameter values can produce saddle-node bifurcation because the system displays two stable steady states and one unstable steady state, which is a typical requirement for bistability of biological switches. A phase plane with the *u* nullcline and *v* nullcline is shown in Figure 4; the intersection points from these nullclines are the steady state points of the system. From the vector field or flow of the trajectories, we can see two stable steady states as attractors and one unstable steady state as repeller.

Figure 4. A phase plane of the *v-u* axes. The *u* nullcline defined by d*u*/dt=0 (thick solid line) intersects with the *v* nullcline defined by d*v*/dt=0 (dotted line) at three points and these points are called steady states (or fixed points). Two of the steady states are stable steady states represented by black dots (0.107, 2.99) and (2.99, 0.107), and these two points are basin of attractors; however, one unstable steady state is denoted by an empty dot (1.164, 1.164) in the middle, and this point is a repeller. Copyright © 2013 Society for Industrial and Applied Mathematics. Reprinted with permission. All rights reserved.
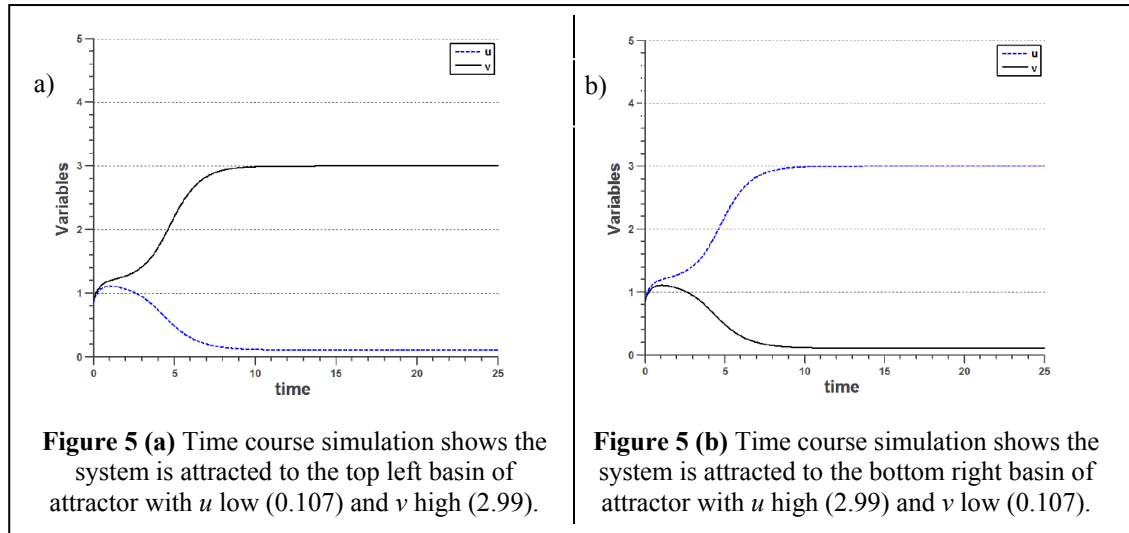
There is a separatrix (light blue line) in Figure 4 that separates the two basins of attractors. The initial conditions decide which basin of attractor a trajectory will be moving towards and thus which protein is turned on (with high protein levels). To illustrate an instance of bistability: for one set of initial conditions u_1=0.84 and v_1=0.87 (Figure 5 (a)), the system is attracted to the top left basin of attractor with *u* low (0.107) and *v* high (2.99); and for another set of initial conditions u_2=0.87 and v_2=0.84 (Figure 5 (b)), the system is attracted to the bottom right basin of attractor with *u* high (2.99) and *v* low (0.107). The qualitative analysis

of a biochemical switch generally involves drawing the nucllines of a two variables system in the phase plane and to identify the steady state solutions as in Figure 4. This concept is generalisable for a system that has more than two variables and the phase plane may not be drawn; however, a bifurcation diagram can be used to capture the bistability and that is why bifurcation diagram is such a powerful mathematical tool (Tyson et al., 2001).

3.2. Time course simulations

Time course simulations are important in generating diagrams for visualizing the variables (either protein or mRNA levels) in the system over time and comparing its dynamics with data gathered from experiments. As explained in the previous section, two slightly different initial conditions can lead to different results: Figure 5 (a) shows that gene V (black solid line) gets turned 'ON' and Figure 5 (b) shows that gene U (blue dashed line) gets turned 'ON' instead.

Figure 5 (a) Time course simulation shows the system is attracted to the top left basin of attractor with *u* low (0.107) and *v* high (2.99).

Figure 5 (b) Time course simulation shows the system is attracted to the bottom right basin of attractor with *u* high (2.99) and *v* low (0.107).

3.3. Drawing saddle-node bifurcation diagram

For drawing a bifurcation diagram, a software package is normally used. In XPPAUT software tool, there is a link from XPP to AUTO; however, AUTO is a "tricky package" in the sense that bifurcation diagram is not generated automatically (Ermentrout, 2002; Segel & Edelstein-Keshet, 2013). Thus, in this section we focus on discussing the steps in XPPAUT and AUTO in drawing saddle-node bifurcation; however, how it works with the method of continuation, readers are encouraged to follow an introductory course lectures from John J. Tyson titled "A Primer in Bifurcation Theory for Computational Cell Biologists" (Tyson, 2010). Basically, a saddle-node bifurcation is a diagram showing the value of steady states and whether the type of steady states are stable or unstable (y-axis) with respect to the change of a parameter of interest (x-axis). A steady state is also known as a fixed point, which is defined by the solution(s) when the differential equations set to zero(s). When the stability of a steady state changes or a steady state is created or destroyed, there is a qualitative change in the dynamics and thus it is called bifurcation (Strogatz, 1994).

Based on the ODE equations listed in (1) and (2), enter the equations into an ODE file as below:

```
u'=alpha1/(1+v^n)-u
v'=alpha2/(1+u^m)-v
param alpha1=3, alpha2=3, n=3, m=3
@ total=500, xp=t, yp=v, dt=0.01, xlo=0, xhi=4, ylo=0, yhi=4, maxstor=500000
# AUTO stuff
@ AutoXMin=0, AutoXMax=15
@ AutoYMin=0, AutoYMax=15
@ Nmax=1000, NPr=200, ParMin=0, ParMax=20, Ds=0.01
done
```

Once you have saved your ODE file, you can get a bifurcation diagram by following the steps below:

Initial —>Integrate —>Go; Integrate—>Last; (Before the link to AUTO, we need to integrate the equations until steady state is reached by repeating Integrate—>Last a few times) File—>Auto; Run—>Steady State; Grab—>Press Tab to choose α_1=3 (by pressing Enter key in your keyboard); Numerics—>Ds = –0.01 (run continuation in opposite direction); Click Run and you will get a bifurcation diagram as in Figure 6. (In AUTO, LP is an indication of saddle-node bifurcation point and you should get as in α_1=1.918 and α_1=9.986). Why saddle-node bifurcation important is the hysteresis behaviour of 'Going up' and 'Coming down' as a toggle switch will be explained next.
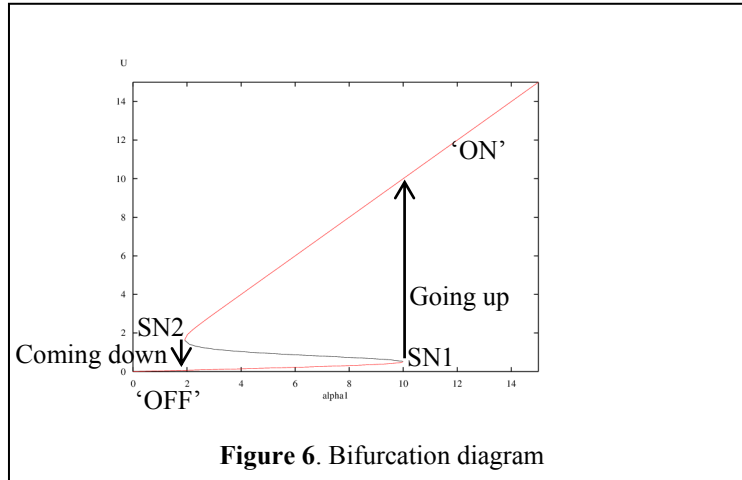

Figure 6. Bifurcation diagram

3.4.   How hysteresis behaviour is seen using XPPAUT simulation

In this section, we will discuss another important concept, namely the hysteresis behaviour that appears in a bistable system. Particularly, we proposed the ways how the hysteresis behaviour which works as memory of the stable steady state can be seen by using XPPAUT time course simulations. From this simple example, bifurcation analysis provides a theoretical exploration of a simple mathematical model constructed with double-negative loops to create a bistable switch. The bistable switch with hysteresis behaviour ensure that when u gets turn 'ON' at the first critical value of α_1=9.986 (SN1) it commits to the decision to stay 'ON' in an irreversible manner as indicated by 'Going up' and stayed at the high stable steady states (Figure 6). To return to the low stable steady states 'Going down' or to get u turn 'OFF' it requires another critical value which is much lower at α_1=1.918 (SN2). In order to obtain these time course simulations from XPPAUT, you need to start with a specific initial condition and integrate the system of equations. Then change the parameter value and follow by integrating the system of equations using the following XPPAUT command:

InitialConds—>Integrate—>Last, which uses the end point of the previous integration as the initial point of the current integration (with this procedure being done repeatedly, the stable steady state of 'Going up' flip to 'ON' or 'Going down' flip to 'OFF' happen when the parameter crosses the critical value of the bifurcation); in this way the memory or state of the system is retained. Thus, it creates a bistable switch. This bistable switch demonstrates hysteresis property; it has memory and tends to stay in either 'ON' or 'OFF' state.
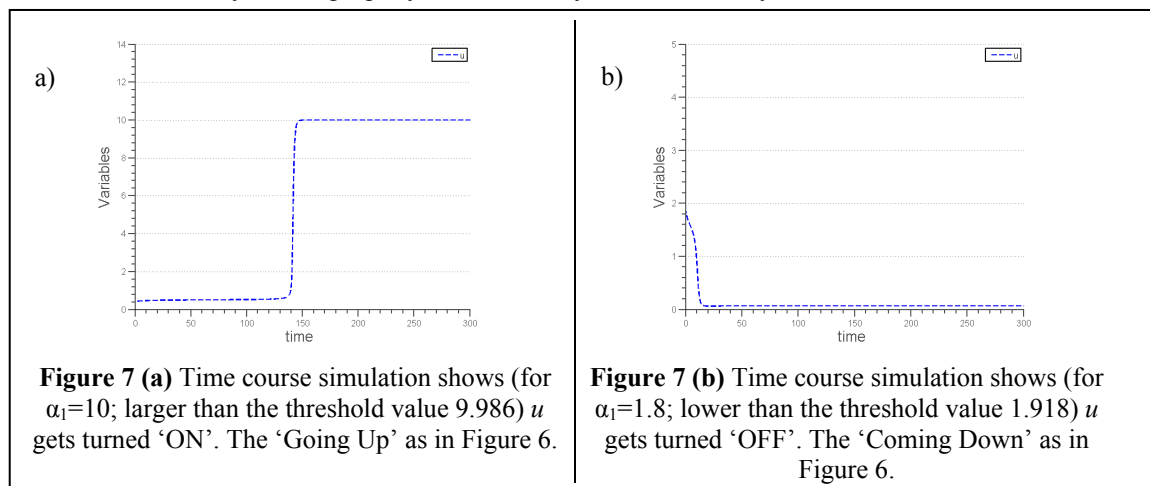

| **Figure 7 (a)** Time course simulation shows (for α_1=10; larger than the threshold value 9.986) u gets turned 'ON'. The 'Going Up' as in Figure 6. | **Figure 7 (b)** Time course simulation shows (for α_1=1.8; lower than the threshold value 1.918) u gets turned 'OFF'. The 'Coming Down' as in Figure 6. |

4.   DISCUSSION AND CONCLUSIONS

In this paper, we reviewed the computational techniques used in theoretical biology for modelling biological switches. First, we discussed the concept of bistability captured by a saddle-node bifurcation. Then, we illustrate the concept of bistability and hysteresis behaviour using a classic synthetic genetic circuit example from Gardner et al. (2000). The bistability is captured by a saddle-node bifurcation diagram.

The use of mathematical models for conceptual study of biological system plays an important role in biology for better understanding of the mechanism underlying the system. In particular, we discussed a simple example of the use of saddle-node bifurcation that has been applied to predict the precise hysteresis behaviour in a synthetic gene circuit (Gardner et al., 2000). These conceptual modelling techniques can be a very useful tool to model ODE of more complex systems. One successful case from literature how these techniques have been used by Novak and Tyson in making accurate prediction of hysteresis behaviour in frog egg cell cycle (Novak & Tyson, 1993), which was later verified experimentally by two independent studies (Pomerening et al., 2003; Sha et al., 2003). For new biological problem, for example, we think that these techniques can be used to explore an open question from recent experiments showing that a bioelectric signal can perturb the system state from normal to disease state of tumour growth (Levin, 2013). It is much like a switch-like behaviour. In conclusion, conceptual modelling will remain a powerful tool that can be employed to enhance our current understanding of biological systems that work as switches to make decisions.

REFERENCES

Ermentrout, B. (2002). *Simulating, analyzing, and animating dynamical systems : a guide to XPPAUT for researchers and students*. Philadelphia: Society for Industrial and Applied Mathematics.

Gardner, T. S., Cantor, C. R., & Collins, J. J. (2000). Construction of a genetic toggle switch in Escherichia coli. *Nature, 403*(6767), 339-342.

Goldbeter, A. (2002). Computational approaches to cellular rhythms. *Nature, 420*(6912), 238-245.

Levin, M. (2013). Reprogramming cells and tissue patterning via bioelectrical pathways: molecular mechanisms and biomedical opportunities. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 5*(6), 657-676.

Novak, B., & Tyson, J. J. (1993). Numerical analysis of a comprehensive model of M-phase control in Xenopus oocyte extracts and intact embryos. *Journal of cell science, 106*(4), 1153-1168.

Pomerening, J. R., Sontag, E. D., & Ferrell, J. E. (2003). Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nature cell biology, 5*(4), 346-351.

Segel, L. A., & Edelstein-Keshet, L. (2013). *A Primer on Mathematical Models in Biology*: SIAM.

Sha, W., Moore, J., Chen, K., Lassaletta, A. D., Yi, C.-S., Tyson, J. J., & Sible, J. C. (2003). Hysteresis drives cell-cycle transitions in Xenopus laevis egg extracts. *Proceedings of the National Academy of Sciences, 100*(3), 975-980.

Strogatz, S. H. (1994). *Nonlinear dynamics and Chaos : with applications to physics, biology, chemistry, and engineering*. Reading, Mass.: Addison-Wesley Pub.

Tyson, J. J. (2010). Lectures: A Primer in Bifurcation Theory for Computational Cell Biologists Retrieved from http://www.faculty.biol.vt.edu/tyson/lectures.php

Tyson, J. J. (2011). Cell Cycle Vignettes Analysis of Cell Cycle Dynamics by Bifurcation Theory.

Tyson, J. J., Chen, K., & Novak, B. (2001). Network dynamics and cell physiology. *Nature Reviews Molecular Cell Biology, 2*(12), 908-916.