End Semester Project
CHM445: Systems Biology

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Introduction

The paper that was used for this project is titled as: *"A Multi-Functional Synthetic Gene Network: A Frequency Multiplier, Oscillator and Switch"* which was published in PLOS One journal on February 17, 2011.

The authors outlined the paper as follows: They first discussed the network design and the conceptual basis for its function as a frequency multiplier and presented the model used to represent the network. Simulations demonstrating the frequency multiplication behaviour followed, and a bifurcation analysis of the model was presented to explain the mathematical basis for its behaviour. The multi-functional nature of the network is then discussed and related to the bifurcation analysis. Although the authors discussed the potential for an in vivo implementation, the project wouldn't discuss that but will present everything discussed before.

The Model

The network comprises 4 gene types encoding transcriptional repressors (R1, R2, R3, R4). Each of these genes is preset in 2 copies and are regulated by different promoters. There are 6 promoters (P1-P6). The network is as shown in the figure below:

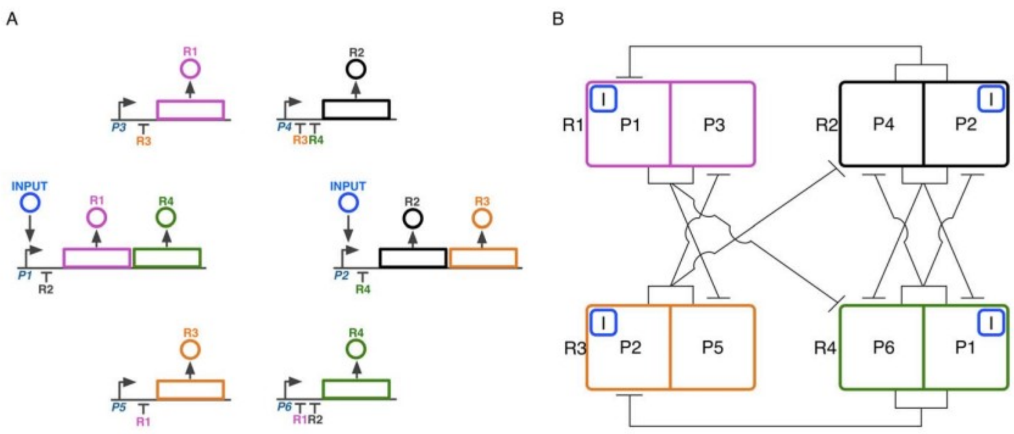


Figure 1. Network design. A. Physical representation. R1–R4 are transcriptional repressors, and P1–P6 denote promoters. 'Input' is a transcriptional activator. Flat-headed arrows represent repression. **B.** Node diagram representation. Each node is a repressor, divided into its two promoter sources. Input is represented by 'I'.

The network is modelled as follows:

$$[\dot{R}1] = a_1 h^+([I])h^-([R2]) + a_2 h^-([R3]) - \delta_{R1}[R1],$$

$$[\dot{R}2] = b_1 h^+([I])h^-([R4]) + b_2 h^-([R3])h^-([R4]) - \delta_{R2}[R2],$$

$$[\dot{R}3] = c_1 h^+([I])h^-([R4]) + c_2 h^-([R1]) - \delta_{R3}[R3],$$

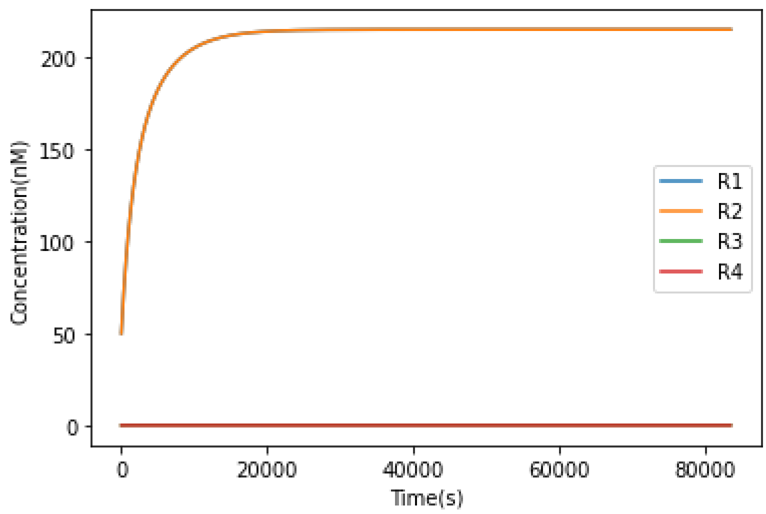
$$[\dot{R}4] = d_1 h^+([I])h^-([R2]) + d_2 h^-([R1])h^-([R2]) - \delta_{R4}[R4],$$

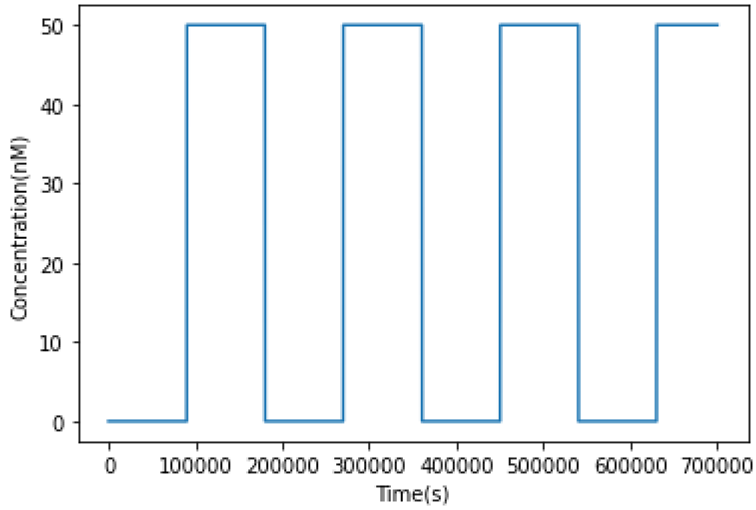
$$h^+([X]) \equiv \frac{[X]^N}{k_A^N + [X]^N} \qquad h^-([X]) \equiv \frac{1}{1+\left(\frac{[X]}{k_A}\right)^N}$$

Reproducing the work

❖ *Fig. 3:*

Figure 3 of the paper tries to produce the **frequency multiplier** behaviour of the network with a square input of *50 nM.* The time period of oscillations is 90,000 seconds. The initial concentrations were R1=R2=50 nM and R3=R4=0 nM. I couldn't get the plot due to the peculiar nature of the *'odeint'* function which uses adjustable time steps instead of the specified time steps. The first plot is for the repressors' concentration vs time, and the second plot is the input signal plot. The result is as follows:

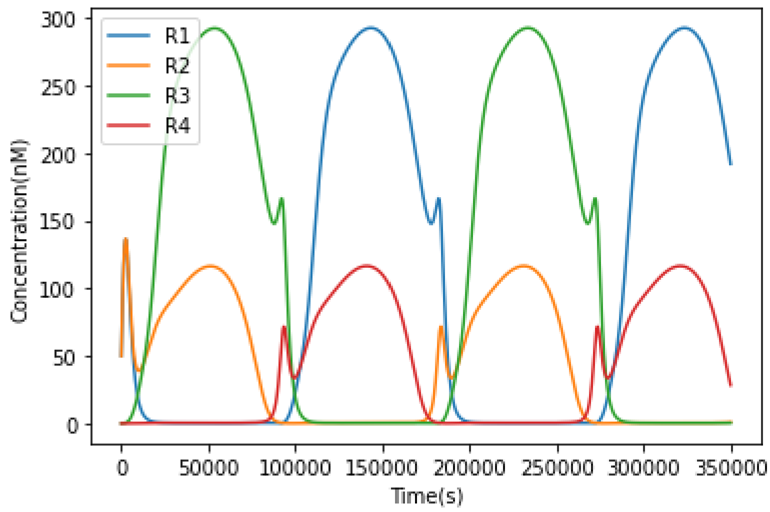


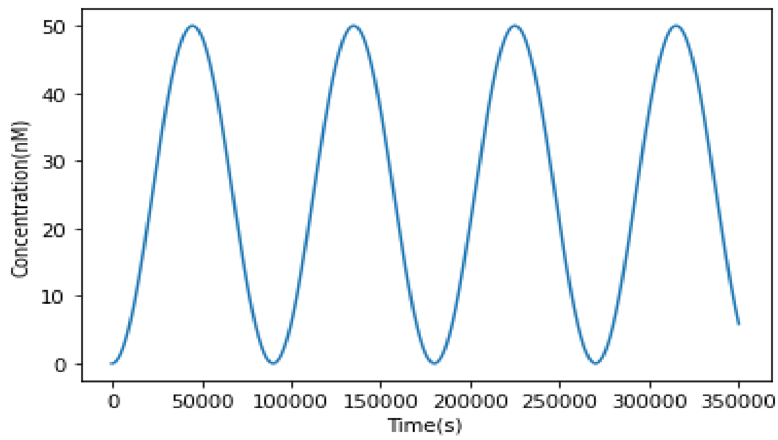


❖ *Fig. S3 supplementary:*

Supplementary S3 of the paper tries to produce the **frequency multiplier** behaviour of the network with a sine input of 50 *nM* amplitude without any offset. The time period of oscillations is 90,000 seconds. The initial concentrations were R1=R2=50 nM and R3=R4=0 nM.

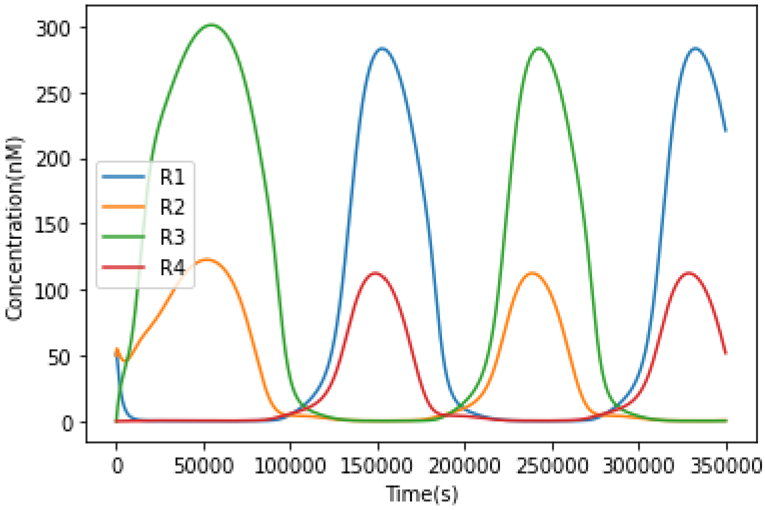
The first plot is for repressors and the second is the signal plot. The result is as follows:

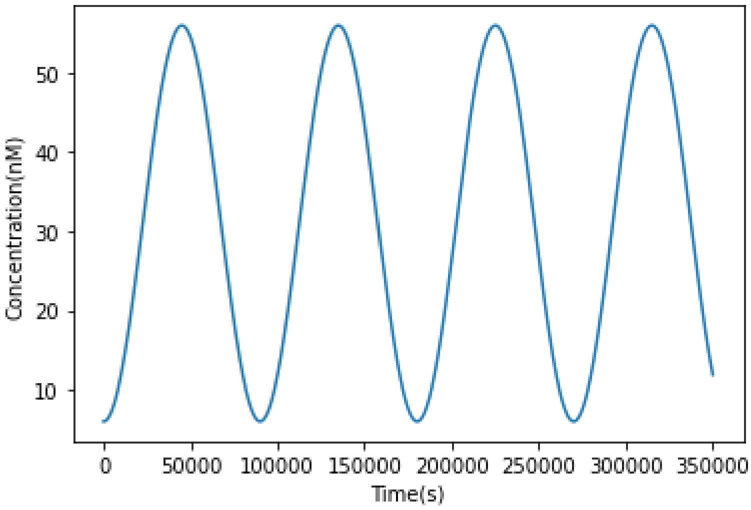




❖ *Fig. 4:*

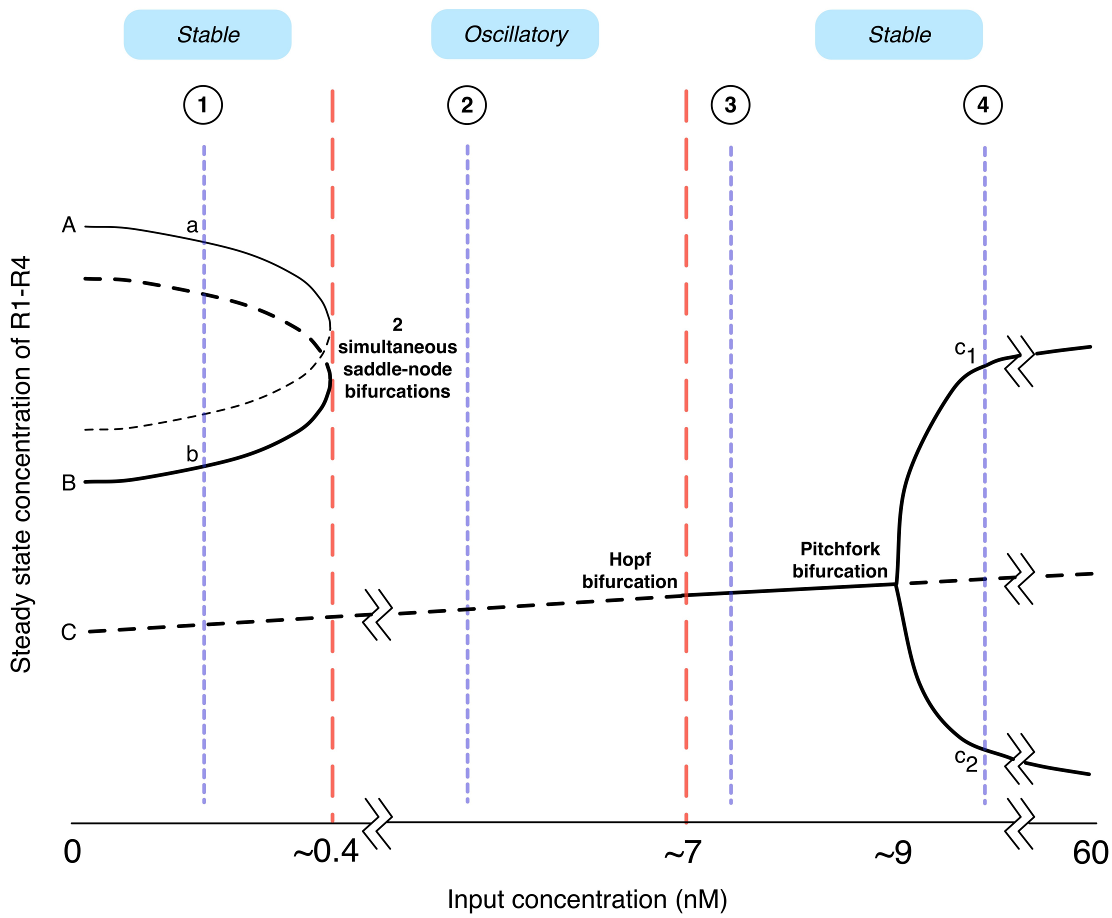
Figure 4 of the paper tries to produce the **frequency multiplier** behaviour of the network with a sine input of *50 nM* amplitude with an offset of *6 nM.* The time period of oscillations is 90,000 seconds. The initial concentrations were R1=R2=50 nM and R3=R4=0 nM.
The first plot is for repressors and the second is the signal plot. The result is as follows:



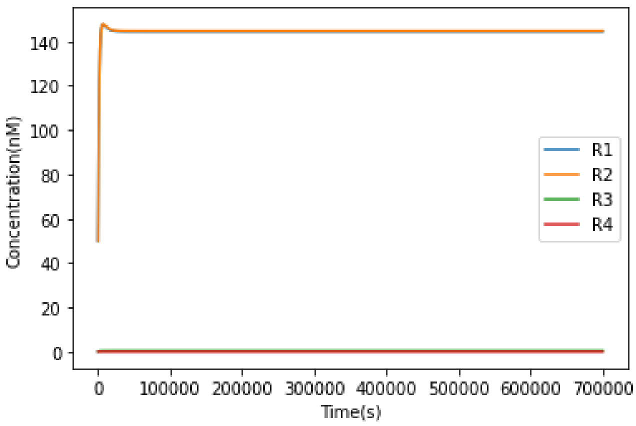


❖ *Fig. 5:*

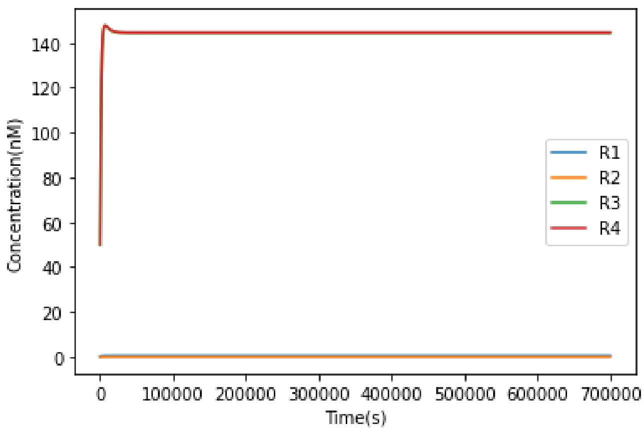
The model behaviour can be divided into four distinct dynamical regions, corresponding to labels 1–4 in figure 5:
1. A region of coexistence of two stable and three unstable equilibria for I = [0, 0.4].
2. A region where a single unstable equilibrium exists, together with stable undamped oscillations emerging from a Hopf bifurcation for I = [0.4, 7].
3. A region where a single stable focus exists, for I = [7, 9].
4. A region of coexistence of two stable and one unstable equilibrium for I = [9, >=60]



Panel 1:

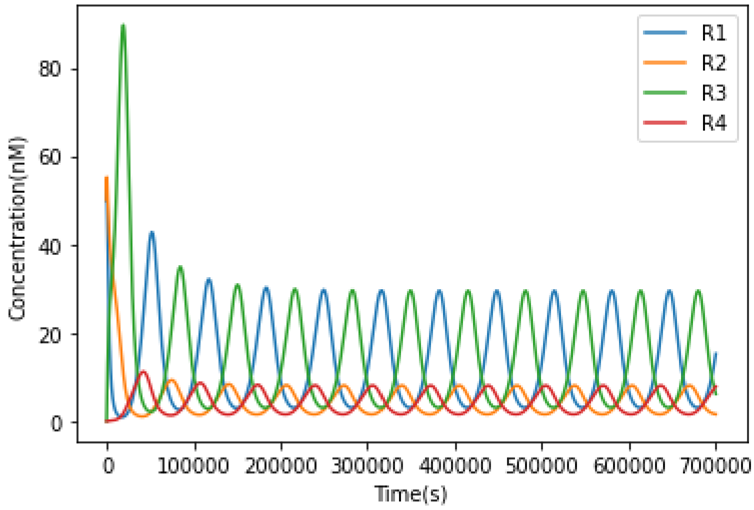


A. => R2(High conc.)



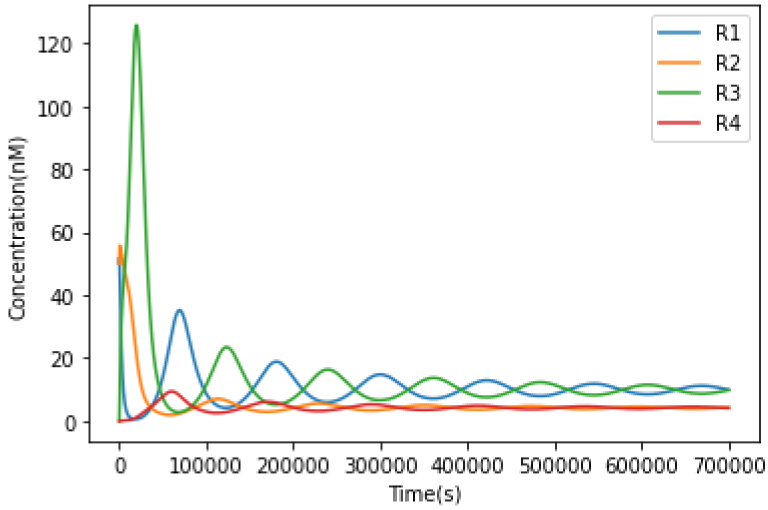
B. => R4(High conc.)

Panel 2:



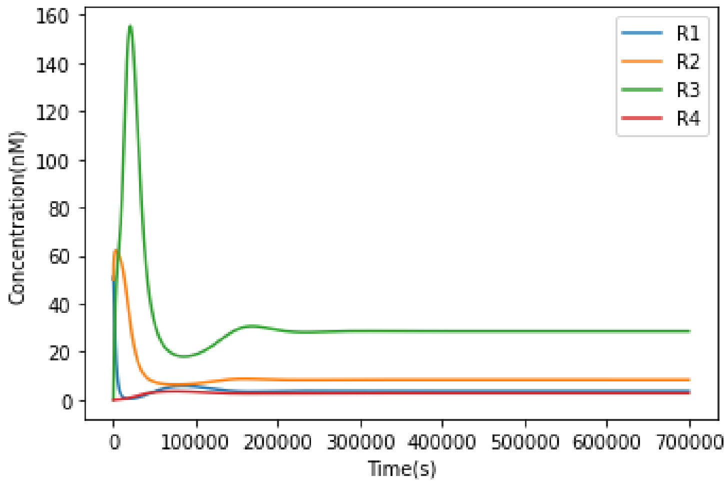
=> Oscillatory behaviour

Panel 3:

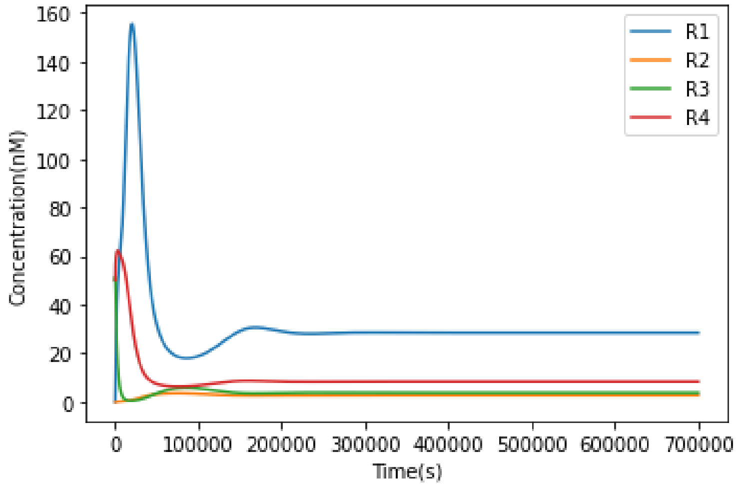


=> Damped Oscillatory behaviour

Panel 4:



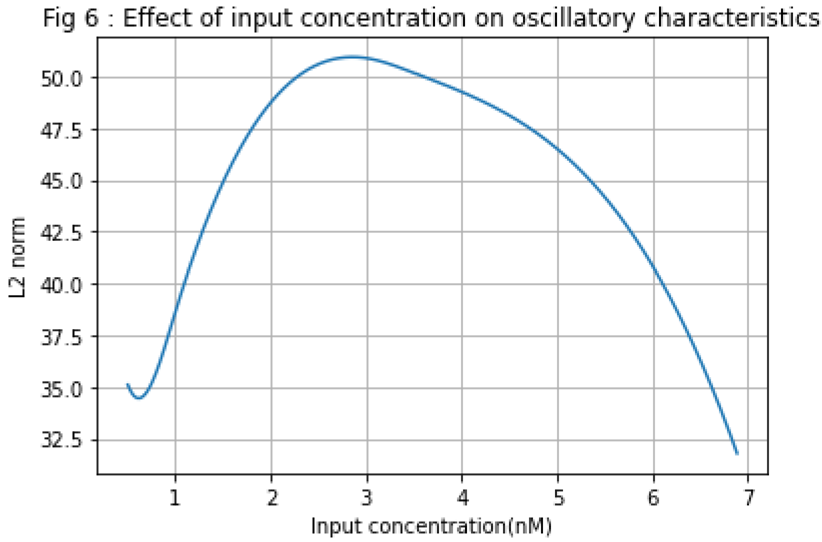
C1. => R3(High conc.)

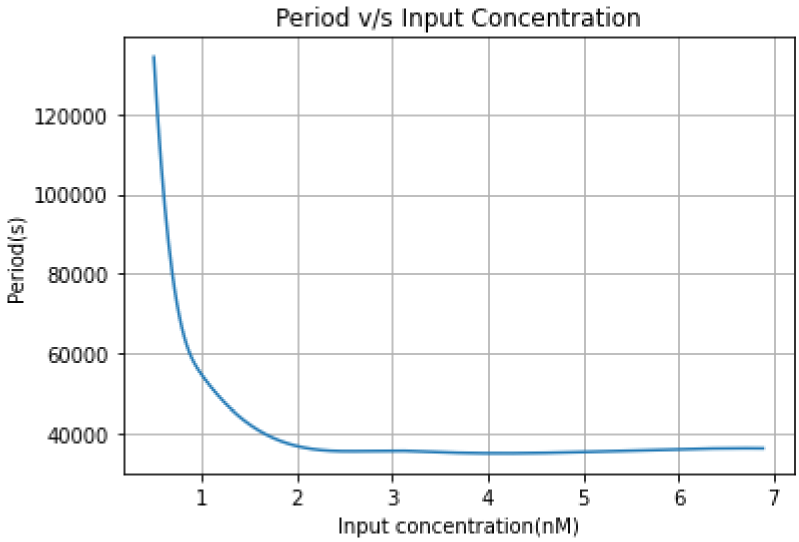


C2. => R1(High conc.)

❖ *Fig. 6:*

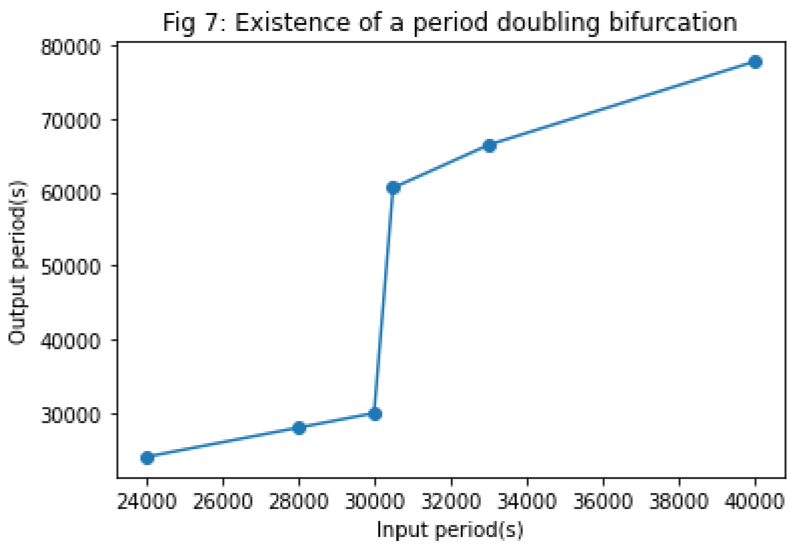
The figure 6 of the paper showcases the effects of input concentrations on the oscillation characteristics. A constant input signal was varied between 0.4 nM and 7 nM at different points and the corresponding oscillation periods and l2 norm for the waves was calculated. L2 norm is the square root of the sum of the squares of wave amplitudes.

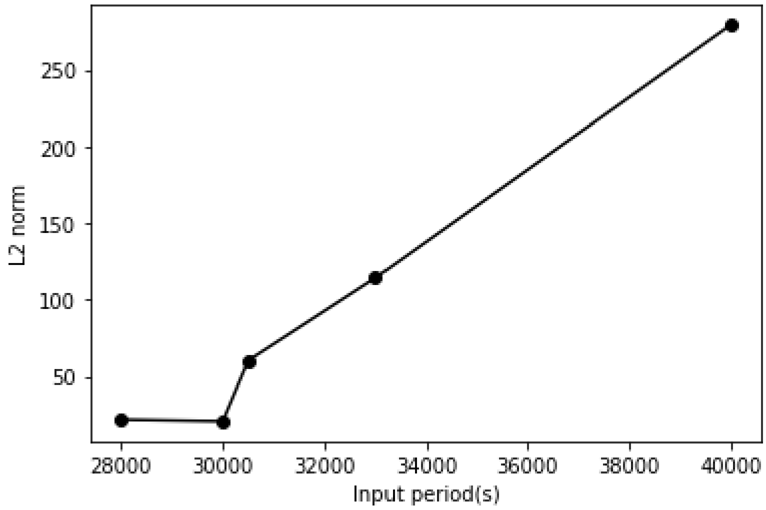




❖ *Fig 7:*

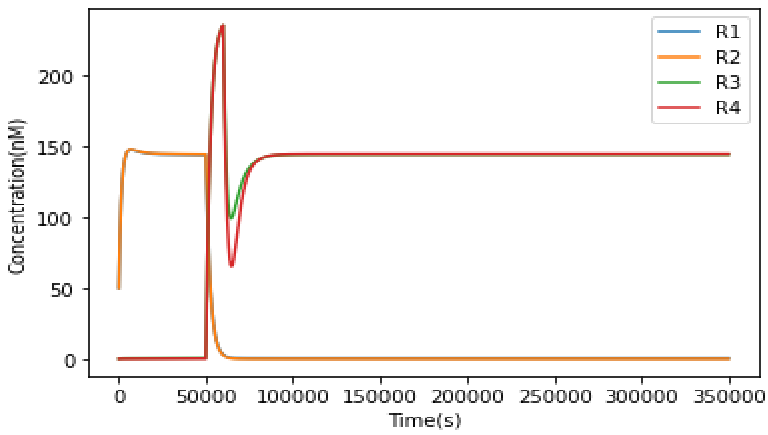
The figure 7 of the paper looks at the period doubling bifurcation. The input signal was *'-A*cos(Beta * t) + A * c',* where A : Amplitude = 50 nM, Beta : 2*pi/p => p is period of input and c : 10^-9. The input period was varied between 28000 and 40000 seconds at different points and the corresponding out periods as well as l2 norm was calculated. I found the period double bifurcation at an input period of **30,500 seconds** as opposed to 27,500 seconds mentioned in the paper. The resulting graphs are as follows:





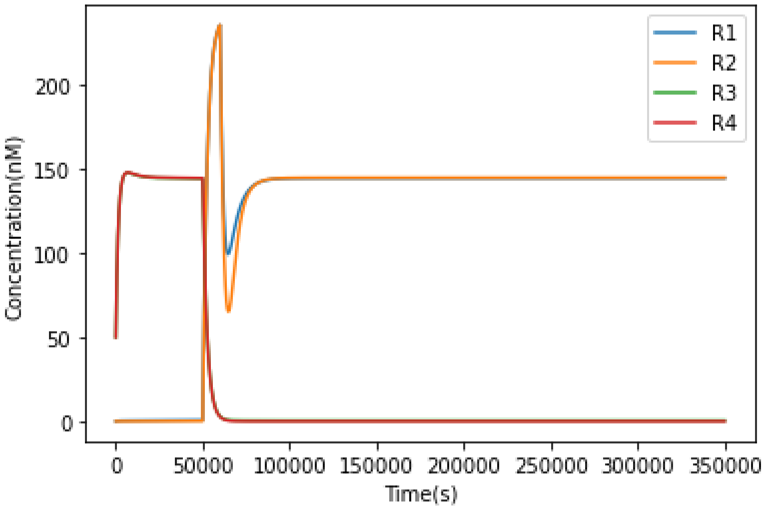
❖ *Fig 8:*

The figure 8 of the paper showcases the switch function of the network. The authors had the switch happen between 150,000 and 155,000 seconds but due to the peculiar nature of the '*odeint*' function, I had to make the switch between 50,000 and 60,000 seconds. The results is as follows:



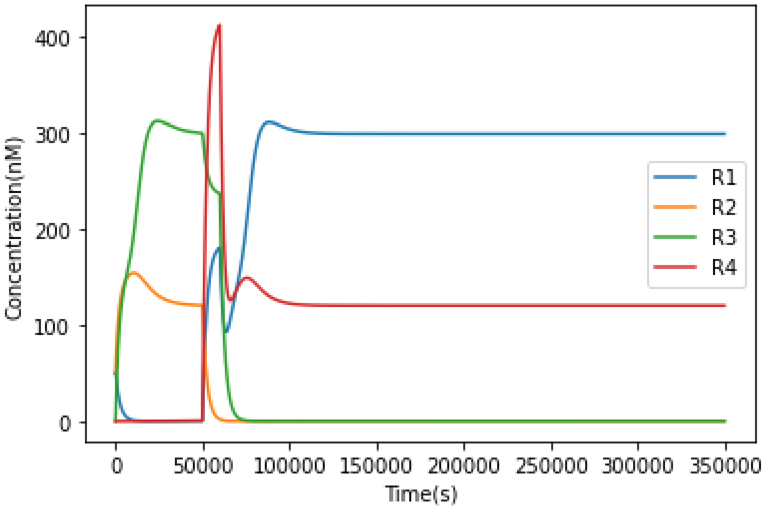
A.

Constant input = 0.1nM ; R1 and R2 high switches to R3 and R4 high



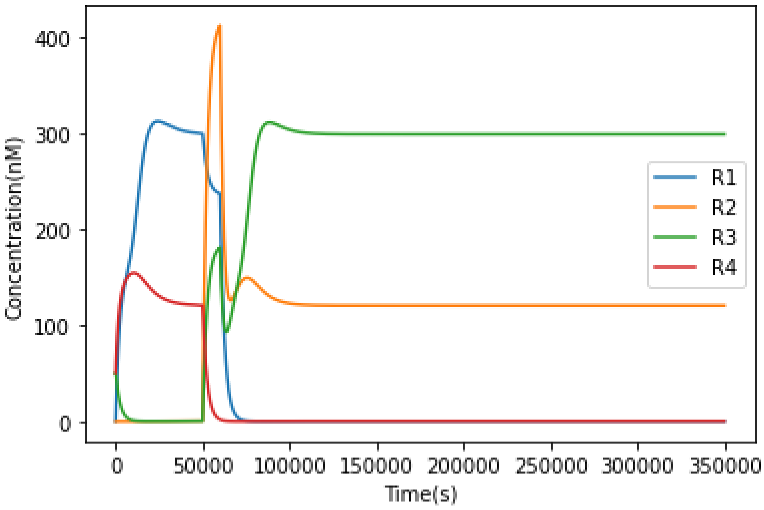
B.

Constant input = 0.1nM ; R3 and R4 high switches to R1 and R2 high



C.

Constant input = 50nM ; R2 and R3 high switches to R1 and R4 high



D.

Constant input = 50nM ; R1 and R4 high switches to R3 and R2 high

Coding script files used for plots

1. Figures 3,4 & 8 as well as the figure in supplementary paper S3 are taken from the python notebook : *‘Switch, Frequency_multiplier&Bifurcation_graphs.ipynb’*
2. Figure 7 is taken from the python notebook : *‘Period-doubling_bifurcation.ipynb’*
3. Figure 6 is taken from the python notebook : *‘Oscillatory_Nature.ipynb’*

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