Generation of modified repressilator network(s) to model effect of light on Circadian rhythm

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Abstract

24-hour circadian rhythms have been observed in most organisms, and a few gene circuits have been identified for the same. One of them is a repressilator at the core of mammalian circadian rhythm, which executes 24-hour oscillations with optimal input from environmental light conditions. However, a variation in physiological responses such as GFR, body temperature, etc have been observed between daylight and nigh-time conditions. This study aims to develop a basic repressilator *in silico* under the control of a singular signal, which when varied would induce changes in either amplitude or frequency of a protein in the repressilator circuit. My hypothesis is that such a system might also exist in circadian rhythms, inducing physiological changes based on signal levels.

Introduction

Data based statistical approaches have led to the discovery of a repressilator system comprising of *cry1*, *per2* and *rev-erb-α* at the core of the Mammalian Circadian Rhythm (MCR) [1]. *In silico* modelling of repressilator networks have shown steady oscillations under a wide range of parameters, with a wide range of models. It should be noted that 2 nodes forming the MCR-GRN are directly affected by light conditions. Recently, a repressilator network called CRISPRlator [2] was constructed, and was shown to have stable oscillations with 17-18 hour periodicity, albeit noisy. This was a modular and an improvement on the first synthetic circuit ever made, the repressilator [3].

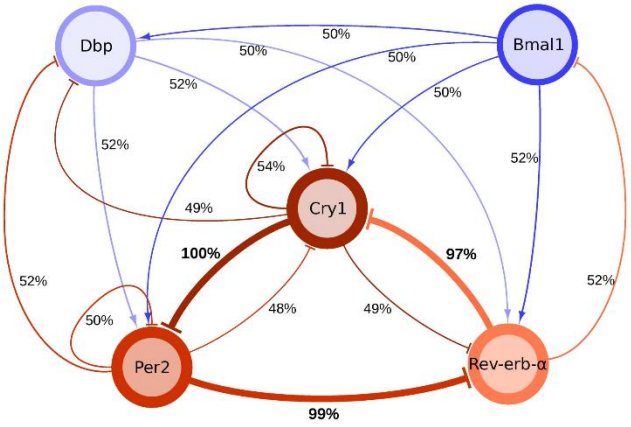

Figure 1: Gene Regulatory Network (GRN) from statistical methods, highlighting strengths of interactions.[1]

Other studies have shown that there are fluctuations in physiological activities like plasma melatonin levels, core body temperature, etc are also modulated based on the time of the day in normal subjects with stable circadian rhythms [4]. Given the correlation between levels of such physiological responses and timepoint during the day, there must be a change in some gene expression patterns that would lead to alteration of downstream processes. Such a change can happen due to multiple reasons, however, a change in the amplitude or frequency of oscillations seems a good starting point. This hypothesis would be supported by studies that have show a change in the circadian rhythm frequency for people suffering from blindness/subjected to abnormal lighting conditions (such as 6-months winter at the poles) [5],[6]. All these conclusions place strong confidence in the hypothesis.

Results

Simulations and modelling of repressilator

Following initial studies by Elowitz, similar ODEs were constructed to model the repressilator. It should be noted that these ODEs are highly simple, and while this solves the issue of complexity, large parameter space and simulation times, it might not be representative of actual activity at all. The ODEs utilized are as follows:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1+p_j^n}, \qquad \frac{dp_i}{dt} = -\beta(p_i - m_i)$$

Where m_i is the mRNA levels of protein i, under repression from protein j (as indicated by the hill function). It should be noted that i, j move cyclically across the three nodes, represented by A, B and C. Simulating this network for different alpha and beta values yields a multitude of oscillations, depicted in Figure 2 (top).

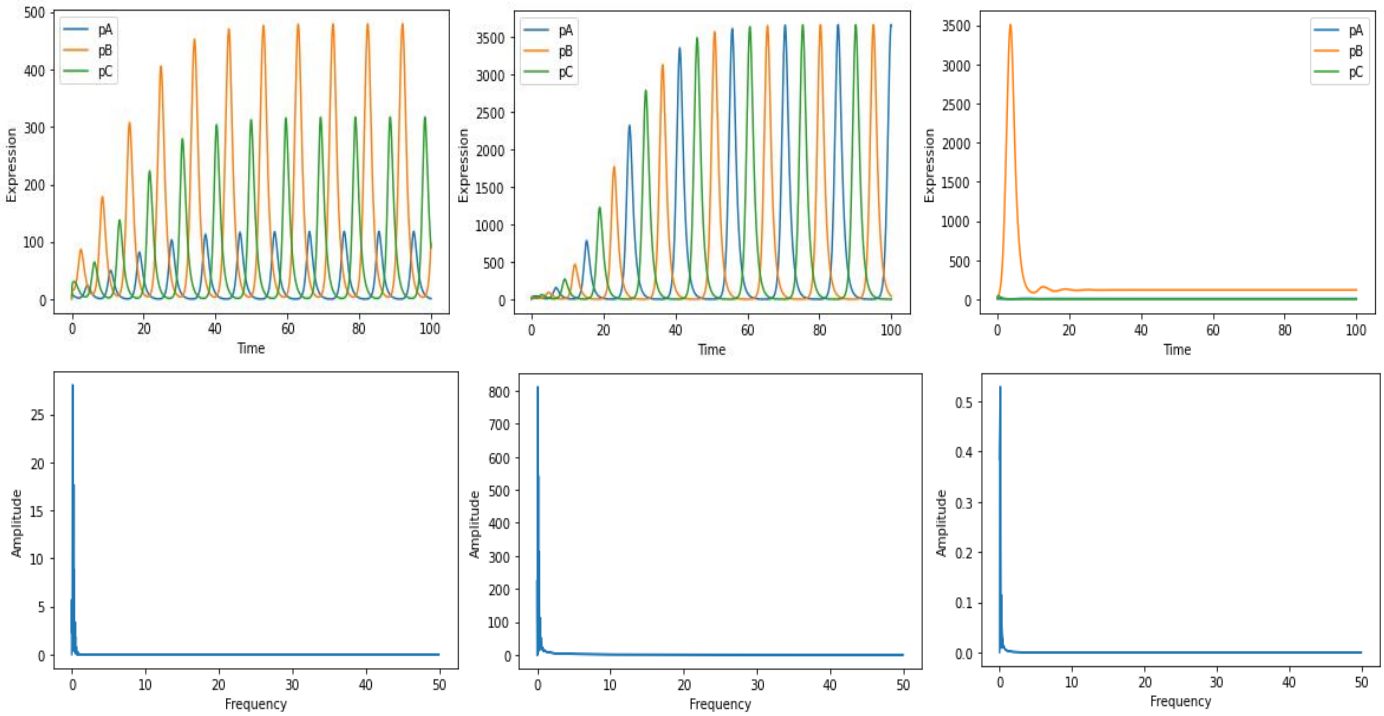

Figure 2: (top) protein expressions of repressilator under various parameters. (bottom) Corresponding Fourier transforms.

As can be observed, steady oscillations with clearly varying frequencies can be observed. Since alpha is the main parameter being changed here, this is a good direction for the hypothesis. Comparing Figure2 (top left) and (top centre), we can clearly see the difference in frequency. The amplitudes are also affected, most probably due to the asymmetric parameters in the first scenario. Hence a scheme evolving a basic repressilator by adding more genes or signals *in silico* could give us an altered repressilator that can be someday constructed using CRISPR-a and CRISPR-i [7].

Measuring frequency and amplitude of basic repressilator

At the first go, the most obvious approach to calculating periodicity of any waveform would be the Fourier transform. Hence, Fourier transform was performed for repressilator under various conditions (Figure2 (bottom)), and the peak magnitude frequency was selected as the base frequency. Since the ODE are not sinusoidal in nature, we can expect that the waveforms of protein and mRNA expressions would also not be sinusoidal. As expected, the approach outlines above resulted in large errors, with deviations as large as 30 percent observed for various parameter sets. Hence a different approach was needed.

Here, the peak values were taken, and the regions above 95 percent peak height were isolated. Next, a sliding algorithm was applied on the isolated peaks to find the peak point (due to computational limits, only max cannot be considered). Then the average times between found peaks was taken to find the periodicity. The suggested method worked and was benchmarked to be used for finding frequencies of any waveforms henceforth.

Checking variation of frequency and amplitude with change in production levels

Before going ahead with checking all combinations of single signal affected, a basic test regarding the validity of hypothesis could be seen by changing the production value of a protein and looking for variations in amplitude and frequency. By varying α_A over a large range, the following plots were obtained

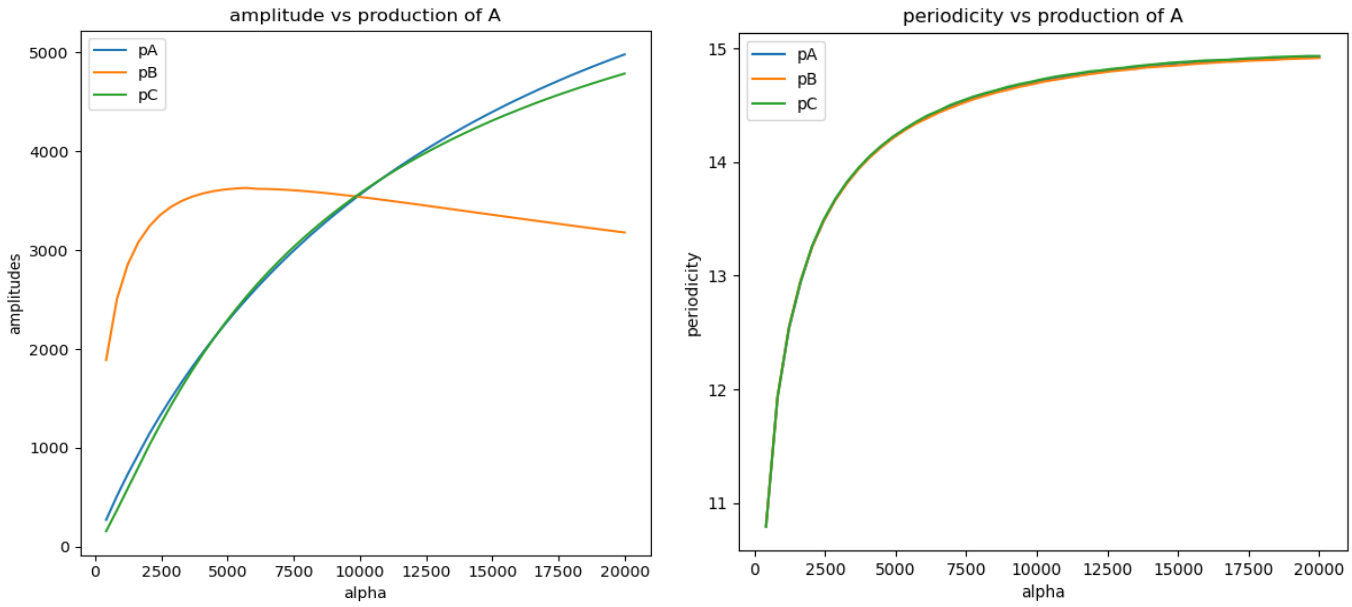

Figure 3: (left) Amplitude of rep proteins vs production values of A; (right) time periods of rep proteins vs production values of A

It was observed that oscillations were upheald for a large range (amplitude > 1000), and all three proteins were synchronised in terms of their freqeuncies. However, the amplitudes of protein B were not in sync with that of the other two, and this could be accounted to the incoherency between B and the coherent nature of A and C (in the forward direction). Similar analysis with changing degradation rates revealed similar, more robust patterns without introducing an incoherency. It should be noted the initial repressilator used here is symmetric in its production and degradation rates. Similar results for assymetric repressilators can be seen in supplementary. Perturbing alpha builds the base on which we shall place our next section.

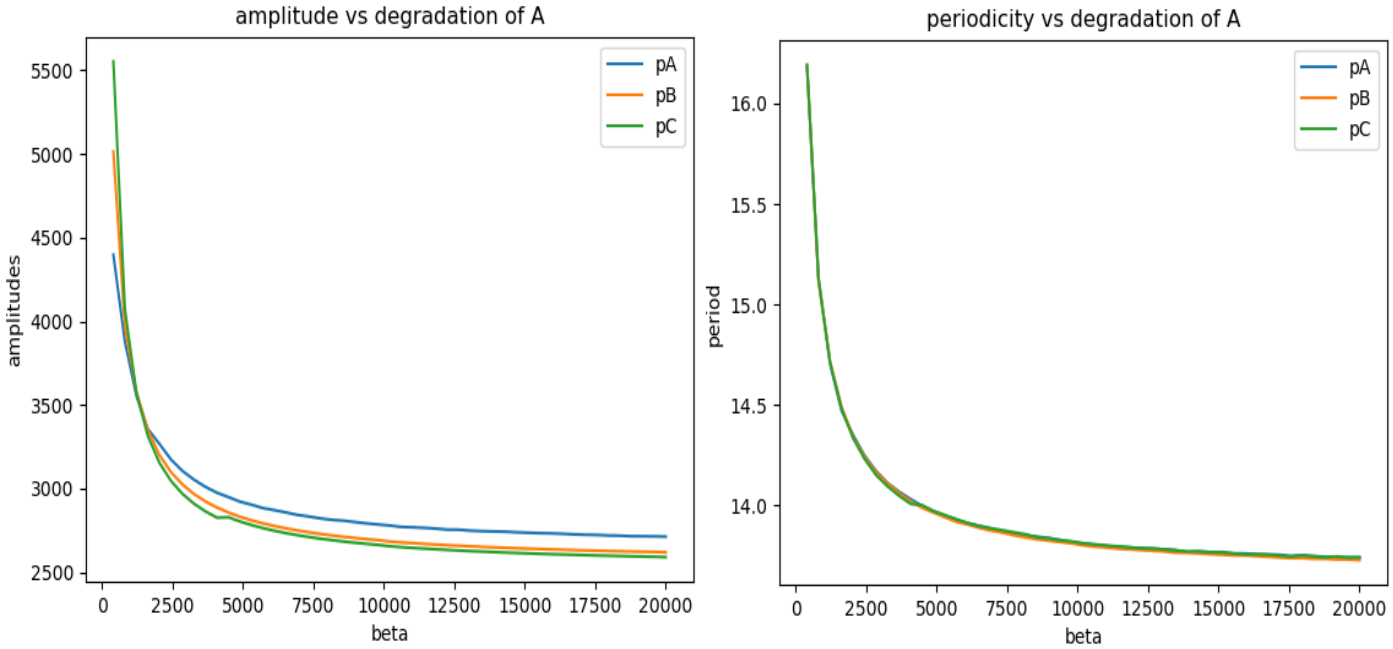

Figure 4: (left) Amplitude of rep proteins vs degradation values of A; (right) time periods of rep proteins vs degradation values of A

Making repressilator affected by single signal and subsequent analysis

Now that we know affecting production rate of a single protein can in effect change its waveform expression profile, we could manipulate our network to do the same. For the purposes of the study, a constant expression signal “sig” was used, which could either inhibit or upregulate the transcription of repressilator proteins. Thus, we have total 27 perturbed networks possible. Using a simple iterative code, these possibilities were generated, except with a slight change in the ODEs. Here, an extra node “Sig” has been added, which remains constant (environmental variable), and is not affected by other nodes. These 27 networks were simulated over 5 mathematically sensible values of signal expression to see how their amplitude and frequencies would be affected. Figures 5 and 6 depict the change in amplitudes and

periodicities for all 27 networks, for protein C. Here, the network number when converted from decimal to ternary system would give the influences by Signal on existing nodes.

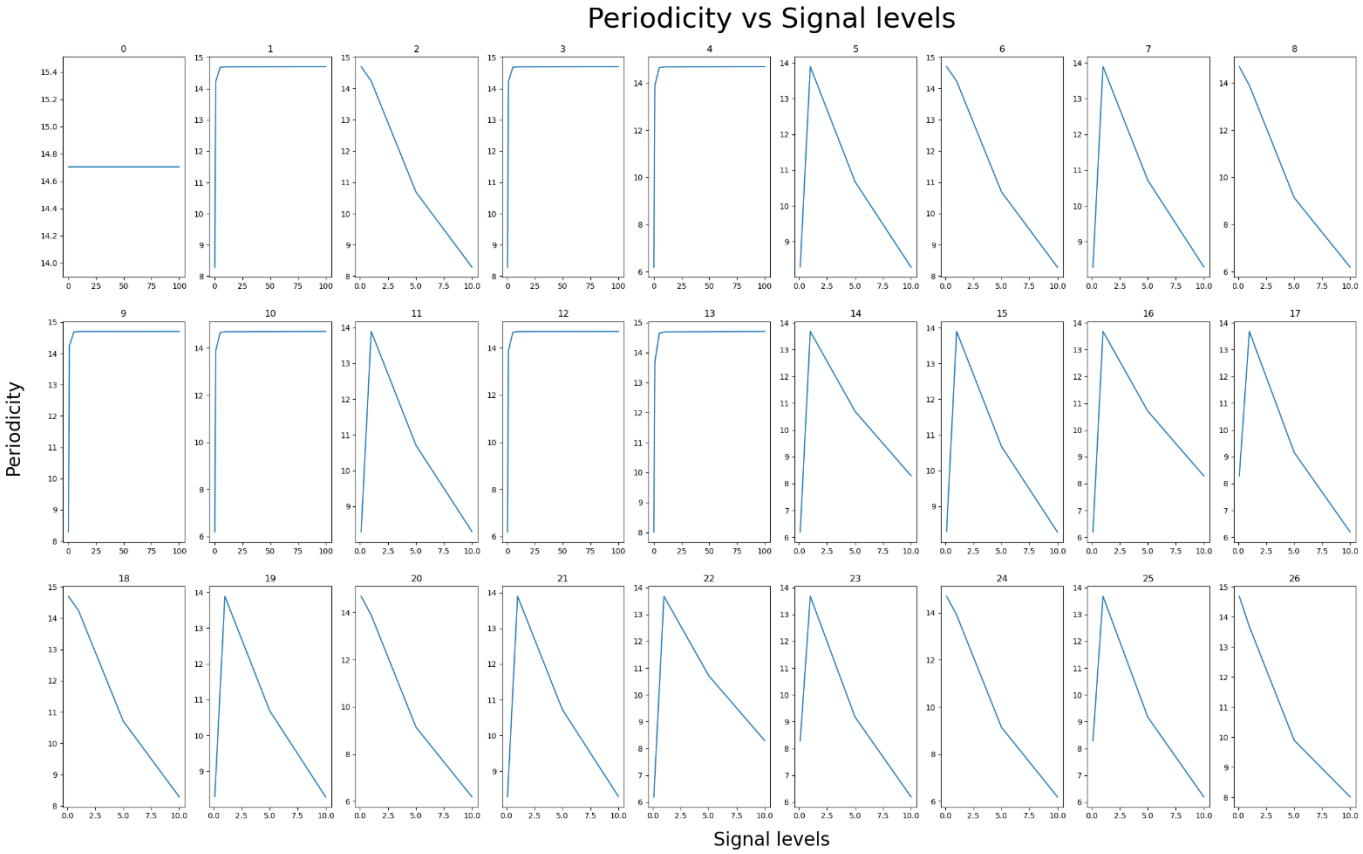

Figure 5: Periodicities of all possible signal repressilators with signal expression values

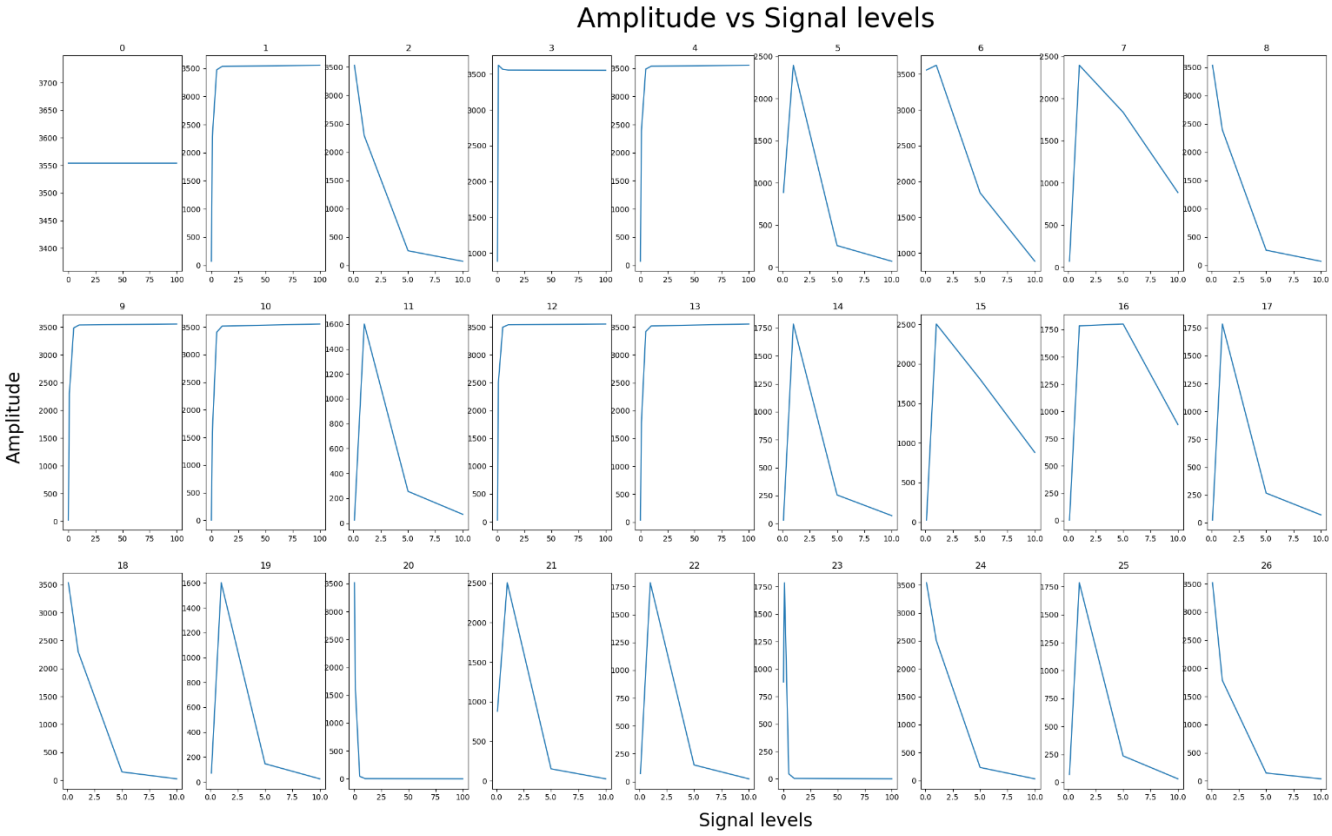

Figure 6: Amplitudes of all possible signal repressilators with signal expression values

From the above graphs, there are multiple things that are apparent. We can clearly see there is a correlation between frequency and amplitude. As one decreases, so does the other. Future continuations of

this study can potentially tackle studying these in detail. Also, we can see that network 2, 6, 8, 18, 24 and 26 show a steady decrease in amplitude and frequency. Hence, we have reached networks that can show dynamic change in frequency and amplitude simultaneously (both are linked, unfortunately), which was the purpose of this study. The supplementary images S2 depict the expression profiles for protein C for the symmetrical repressilator condition for network 18. Asymmetric repressilator also depicted similar results (only periodicity profile attached in S3).

Discussion and Future Directions

This study successfully depicted the possibility of easy dynamic control of frequency/amplitude of a modified repressilator GRN. While there are multiple potential pitfalls to the mathematical formalism and study approach, the study is a good starting point towards understanding the dynamic nature of repressilators.

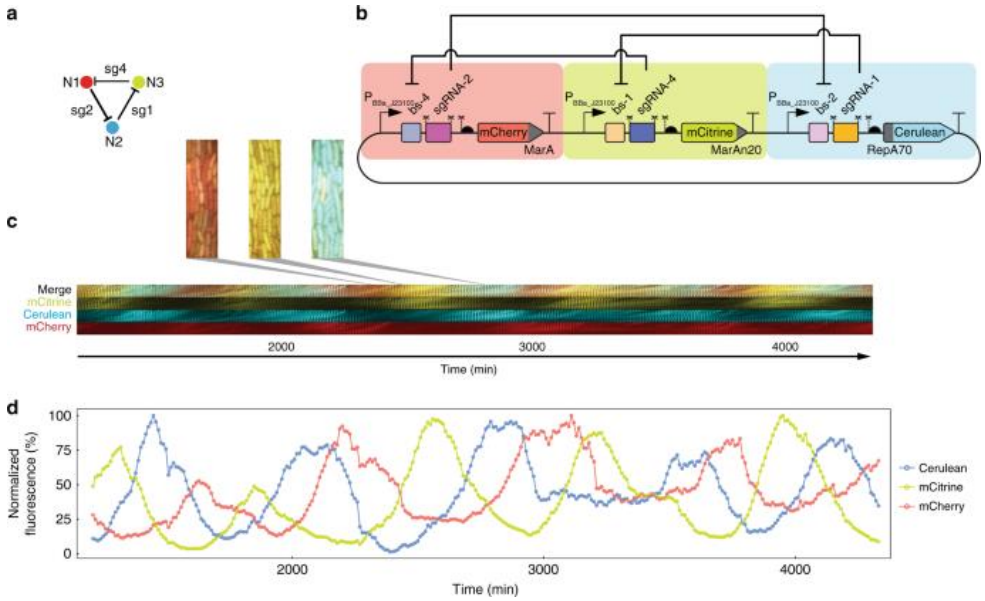

Figure 7: Image from Moreno et al, 2020, depicting CRISPRlator and the expression profile [2]

While the project was conceived form existing studies on Circadian rhythms, there are many interesting and exciting prospects. The existence of a CRISPRlator (Figure 7), gives a practical possibility to this *in silico* study. Existing work has shown the superiority of CRISPR-i and CRIPSR-a over Transcription Factors (TFs) [2],7],[8]. In theory, it seems an easy task to have a scaffold RNA (scRNA) or guide RNA (gRNA) under the control of *lac* operon, pTet, etc which can act as the external signal. Modulating this could potentially result in modulation of expression profile of the CRISPRlator and is certainly a very exciting prospect. The synthetic biology applications are seemingly endless, with repressilator being only a case example for the purposes of the study.

Methods

Python3 was utilised for all coding and simulation needs, with the *scipy* and *numpy* packages playing a huge role. Rest can be seen from the codes available on the GitHub Repository.

Codes and Algorithms

All codes, inputs, outputs, reports and topology files are available at the GitHub repository "Repressilators_in_Circadian", under my profile "Lakshya3141".

https://github.com/Lakshya3141/Repressilators_in_Circadian

Supplementary

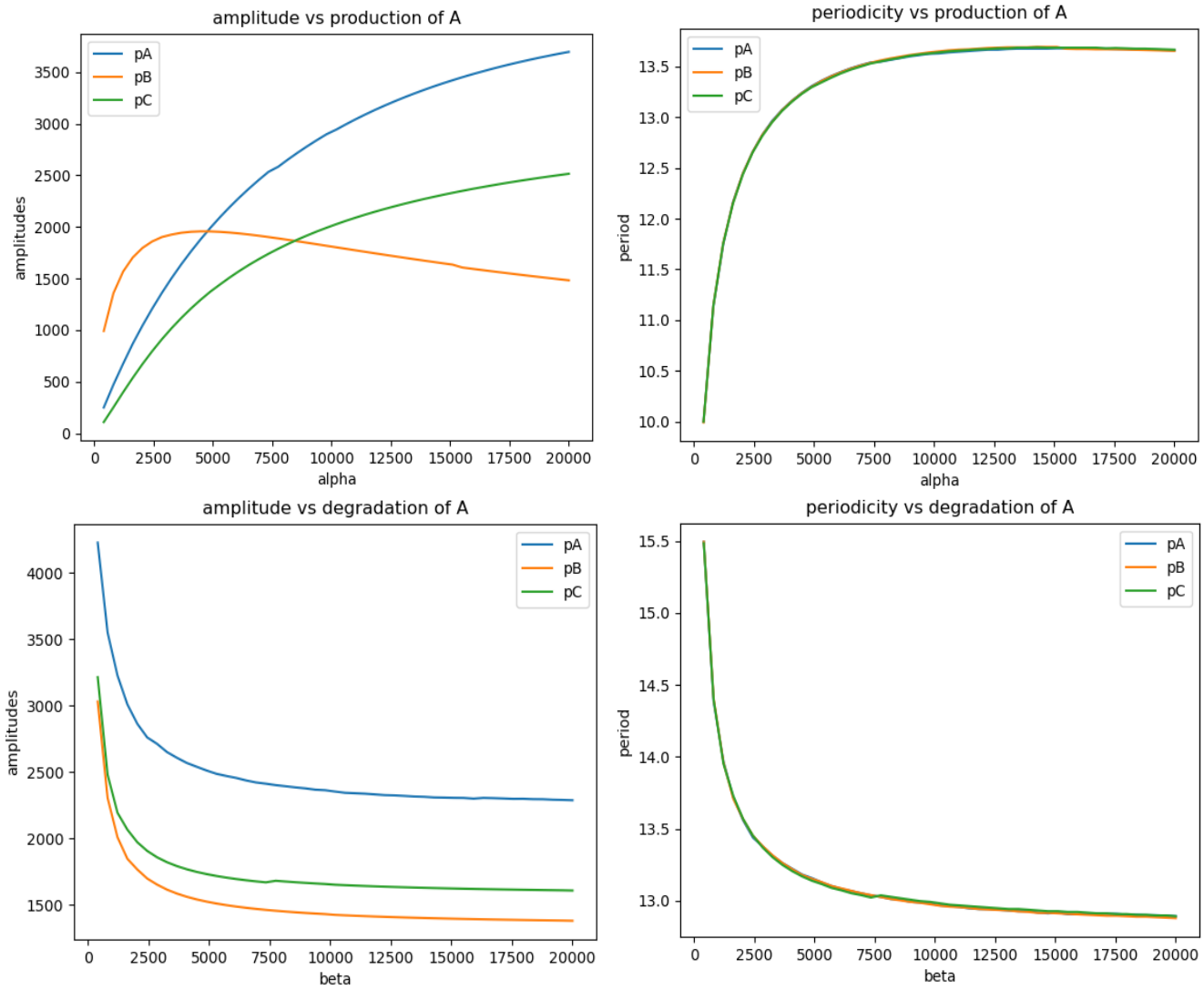

Figure S1: Similar analysis to figure 3 and 4 for an asymmetric repressilator.

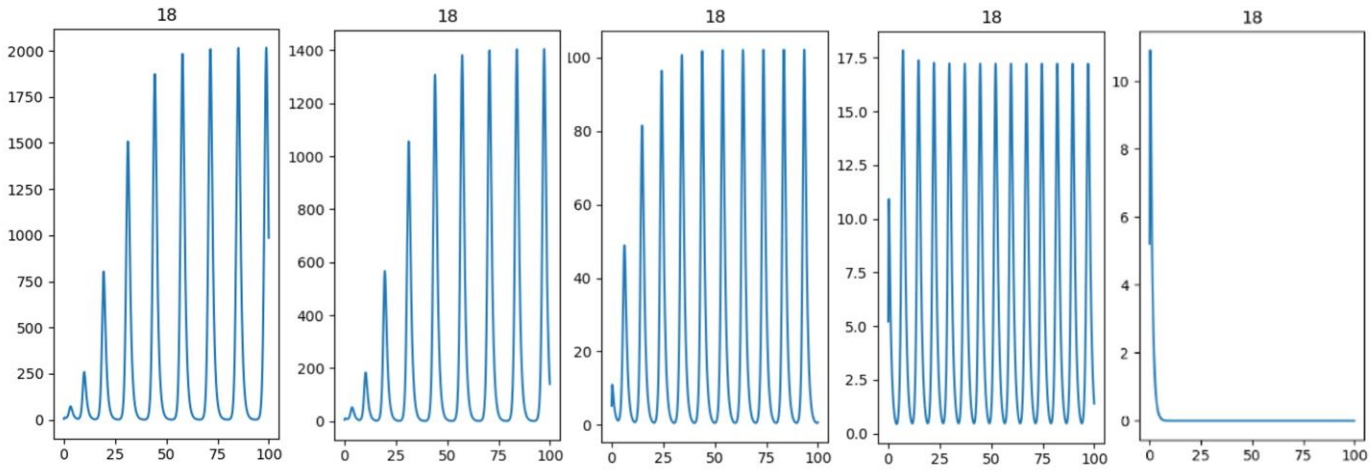

Figure S2: Expression profile of network 18, signal level increases from 0.1 (left) to 10 (right)

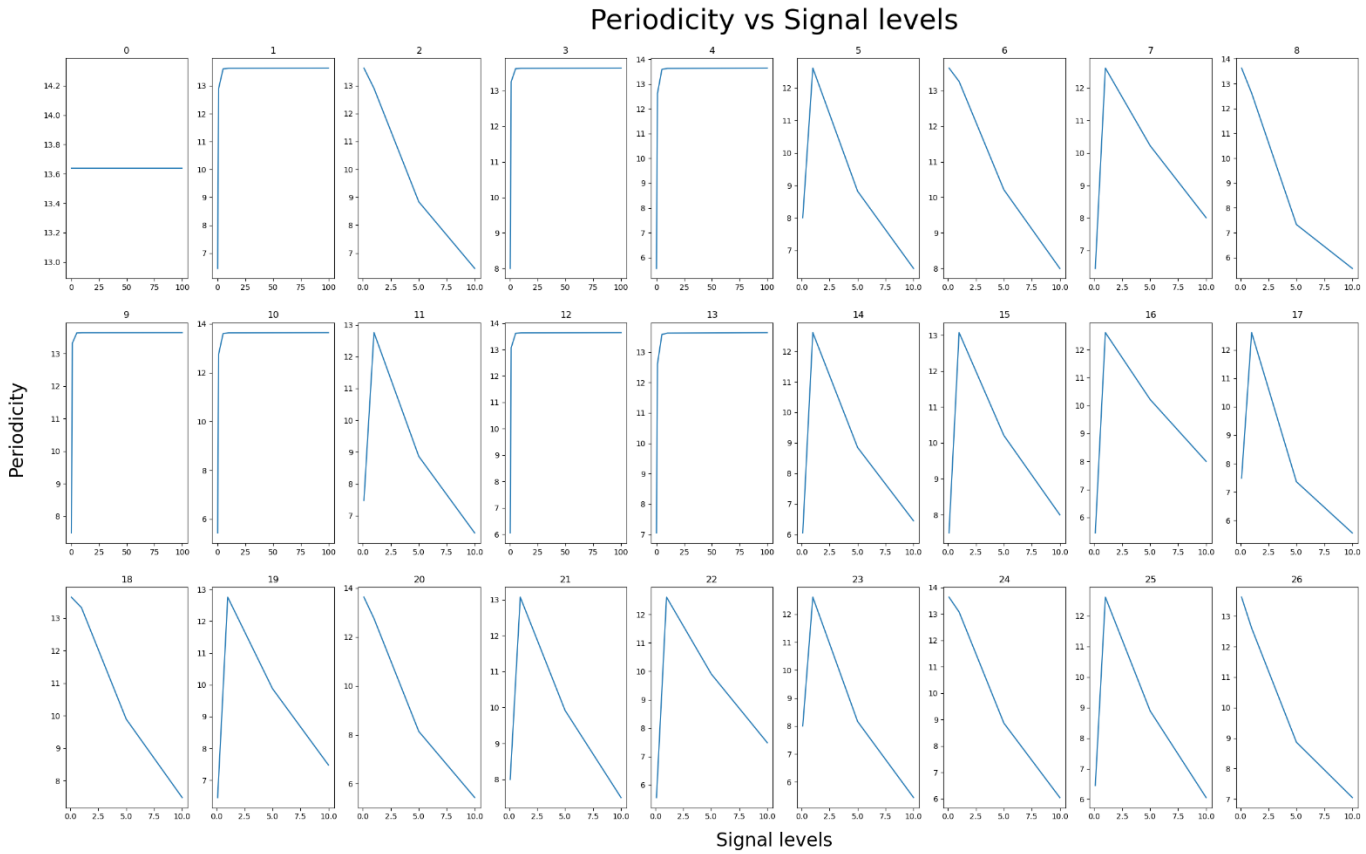

Figure S3: Periodicity vs Signal level for asymmetric repressilator.

References

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