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Comparative Study of Feedback Control System when Designing Synthetic Genetic Oscillator

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***Abstract*— Synthetic biology main focus is to reveal the standard design scheme of natural biological systems through designing and modelling of various synthetic genetic devices or circuits. Gene regulatory network in natural biological systems has been the main reference used as the basic theory when designing genetic device. With the use of various methods, some genetic devices exhibit oscillatory behavior. However, the main problem when designing devices is to ensure its robustness in terms of device performance. The feedback control system is said to be helpful when designing circuit in electrical engineering study. The same type of control system has also been used in designing synthetic genetic oscillatory device. This study briefly discussed on feedback architecture and design approach used when designing synthetic genetic oscillator.**

Keywords — Synthetic biology, genetic oscillator, feedback, synthetic device.

I. Introduction

Synthetic biology is aimed to model and produce new cell through the combination of different fields such as biology, engineering and computer science [1]. With the help of current arising technology, synthetic biology research can discover new cell rarely found in nature [2] through the discovery of new biological parts and also regulatory system. The modular parts in synthetic biology are modelled with referring to the nature biological parts. Starting from the basic deoxyribonucleic acid (DNA) sequence characterized into specific biological parts which exhibit their own function. The combination of several biological parts formed genetic device thus produce a system with several interconnecting devices. Biological part in synthetic biology is referring to fixed nucleotide sequence with distinct essential biological function [3] such as promoter. Genetic device such as oscillator which performs looked-for human-defined function [1, 3] is designed through the arrangement of several biological parts associate to each other [3]. Synthetic biology system comprises of one or more combination of devices which perform specific function such as synthesizing a molecule of interest [1].

In biology, the synthesis of gene regulatory network exhibits such behavior which can be categorized as two genetic devices either switches or oscillators [1]. The transcription process in gene regulatory network can be modelled as genetic single input single output (SISO) device [3]. In transcription-based SISO device, an activator as its input is required to activate the transcription process [3]. However, if the input is substituted with a repressor, the transcription process will be blocked [4]. Repressilators are one of the oscillator types formed through a cycle of repressed gene. Each gene suppresses the transcription of the next gene and is suppressed by previous gene in the cycle [1]. The expression level of each gene gives oscillation behavior since each gene is suppressed by previous gene. The oscillation products (expression level) evolve through time [1, 3, 5]. Based on natural phenomena in gene regulatory network (oscillation behavior), many researchers have studied and worked on designing synthetic genetic oscillator which can function as desired.

The problem appears in designing and modelling synthetic genetic oscillator is device performance [3-4]. As an example, when designing the genetic oscillator with referring exactly to the natural gene regulatory network, the performance is not robust. One of the capabilities in biological systems is their ability to dynamically evolve through time under certain condition and environment [3]. The same theory was used by researchers to ensure the performance of synthetic genetic device in terms of evolutionary robustness. However, the same result cannot be produced if the designing of genetic devices follows exactly the structure of gene regulatory network [5]. The main problem in designing synthetic genetic device is the device performance in terms of evolutionary robustness [6-8].

System control is one of engineering principles that has strong impact in the design of synthetic genetic device [8]. This principle used to regularly automate the regulation process in order to maintain the device specificity performance in limited amount of time. One method in system control is feedback architecture [8-9]. Feedback architecture can be used to prevent noise appearance, decoupling relationship between multiple input and output, and also set the direction of system output [8]. The same control system also exists naturally in biological system. Feedback architecture directs the output of the system to become its input. Some researchers have used feedback architecture when designing synthetic device [6, 10-12]. There are also some researchers apply feedback architecture on several field such as metabolic control [13], biosensor design [14] and population control [15].

This study mainly focusing on the feedback architecture applied during the designing of synthetic genetic oscillatory devices. Feedback architecture is discussed properly including its two types along with its properties. Three different feedback architectures that have been used by previous researchers when designing oscillator are simply explained and compared in terms of their performance, advantages and disadvantages.

II. Feedback Architecture

Feedback architecture is a control system that makes use the output of a system to become its input [8]. This control system gives major impact to automate synthetic genetic devices process. In forward engineering, researchers apply feedback architecture when designing synthetic genetic network in order to get desired network behavior. The use of feedback architecture in synthetic genetic network are predicted to influence oscillatory behavior in the network. There are two types of feedback architecture: positive and negative feedback [4, 8]. In system biology, both types of feedback also exist in various natural biological networks including gene regulatory network. Feedback architecture plays great role in the dynamics, variability and response of the system [8]. Both type of feedbacks are discussed in terms of response time taken, input-output relationship and variability of the system.

A. Properties of Negative Feedback

Negative feedback happens when the output of a system is fed back as input in situation where it reduces the disturbance in the system. In gene regulatory network, negative feedback happen when a regulatory protein often called as transcription factor (TF) binds to certain part on DNA (operator) and down-regulates the transcription process. The TF that down-regulates transcription process usually known as repressor protein.

Natural biological systems have capability to response and adapt to its new environmental condition. Negative feedback architecture enables a system to be able to adapt in new environmental condition in short time [8]. Its system has a tendency to achieved equilibrium in short amount of time. In terms of input-output relationship, negative feedback shows a linearized response between input and output [8]. This would be helpful in synthetic biology since the output of a system can be tuned by varying the input signal to meet expected behavior. Biological system exhibit noise arises by cell-cell variability in protein level and variation in gene expression level [8]. Negative feedback gives advantages by reducing noise and perturbation effect arise in a system.

B. Properties of Positive Feedback

Positive feedback occurs when a small disturbance in a system become the causes for changes in perturbation. Positive feedbacks are greatly used in systems biology. In gene regulatory network, positive feedback occurs when transcription factor up-regulates the transcription process.

A system with positive feedback responses differently than negative feedback to achieved steady-state behavior [8]. It takes up a longer amount of time to adapt to new environmental condition. Aside from that, it said that positive feedback could result in ultrasensitive response in relationship between input and output [8]. Small changes in input signal could give great impact on the output. However, positive feedback is helpful to be used in designing synthetic devices that require switch-like behavior. Lastly, having positive feedback in a system is predicted to increase noise and make the system exhibit bistable behavior [8]. The ultra-sensitivity of the output resulted from variability changes in input that increases the noise. A system consists of strong positive feedback possibly exhibits bistable behavior [8].

III. Design Approaches

A. Negative Feedback

Elowitz and Leibler [10] used negative feedback architecture when constructed simple oscillatory device. The device called repressilator consists of three genes code for repressor protein where each gene represses its successor gene while being repressed by its predecessor gene forming a cycle of repressed gene. From time to time, the repressilator inhibits the synthesis of fluorescent protein readout, GFP. Elowitz and Leibler [10] implemented the design into *Escherichia coli* (*E. coli*). In the result, they found that negative feedback loop can cause the device to exhibit temporal oscillation where the oscillation falls out of phase after several number of cycle.

B. Linked Positive and Negative Feedback

Coupling positive and negative feedback architecture can ensure stable oscillation [5]. Atkinson and his colleague [11] constructed simple oscillation model to observe oscillatory behavior by linked positive and negative feedback together in one circuit. The circuit consists of two genes: one code for activator protein and another one for repressor protein. The activator gene activates the transcription of itself and another gene while the repressor gene represses the transcription of the activator gene. However, the observation of implementation in *E.coli* takes 20 hours, which is long-period of time. Although undamped oscillation cannot be attained, it still can reduce the number of damped oscillation. Tigges and his group [12] also used linked negative and positive feedback when constructing circuit and implemented it in mammalian cell. They use tetracycline-regulated transactivator (tTA) that activates its own transcription (positive auto feedback) and repress its own transcription (negative feedback) mediated by its antisense RNA. Their result indicates that the circuit showed steady cycling and oscillation is tunable.

C. Dual Feedback

The use of dual feedback architecture gives several advantages [4]. Stricker and colleague [6] made use of dual feedback control when constructing oscillatory circuit. The circuit comprises of two genes: activator and repressor. However, these two genes provide dual feedback in the circuit where activator gene activates transcription of both genes in presence of support molecule and repressor gene represses transcription of both genes in absence of inducer. Based on the result, they proved that by using dual feedback control, the circuit can achieve robustness and the oscillatory period is also tunable.

All three approaches used by previous researcher have advantages and disadvantages. Those approaches can be used depending on what particular function that the researcher seek for. Normally, they first determined on what to investigate, then decide on which feedback control need to be used. Table 1 summarized all approaches discussed above including its advantages and disadvantages

TABLE 1: SUMMARY OF ADVANTAGES AND DISADVANTAGES OF DESIGN APPROACHES.

Design Approach	**Related Works**	**Description**	**Advantages**	**Disadvantages**
Negative feedback	[10]	Design consists of three genes code for repressor protein connecting with each other in a cycle (lacI, tetR and cL)	- simple oscillator circuit - can observe in short period of time	- oscillation fall out of phase - oscillation damped out after several cycle
Linked positive and negative feedback	[11]	Design consists of two genes: one code for activator protein and another one code for repressor protein (glnG and lacI)	- simple oscillator - number of damped oscillation is reduced	- observation takes longer period of time
	[12]	Design consists of one gene that activates and represses its own transcription mediated by antisense RNA (tTA)	- simple oscillator - tunable circuit - steady oscillation cycle	- observation takes longer period of time
Dual feedback	[6]	Design consists of two genes: activator and repressor in which auto regulate both gene under control of hybrid promoter (lacI and araC)	- simple oscillator - oscillator performance is robust - tunable oscillator circuit	- activator gene is dependent on support molecule

IV. CONCLUSION

In this study, the feedback architecture functioned as a system control is explained briefly. The properties of negative and positive feedback architectures are discussed in terms of system dynamics, systems variability and input-output relationship. Aside from that, three design approaches used by previous researchers in designing synthetic oscillator devices are discussed in terms of design and performance. Lastly, these approaches are compared in terms of benefit and limitation faced by previous researchers as stated in their works. The summary of the comparisons are listed in table 1 above.

As conclusion, based on the comparisons, all three approaches used previously have something in common. For all approaches, the design architecture is easy to be applied when constructing oscillator. Oscillator consists only negative feedback architecture is proved to consume short amount of time during observation [10]. Previous works by researchers showed that combination of positive and negative feedbacks would result in the decrease in number of damped oscillation and the ability of oscillator to be tuned [6, 11-12]. However, only system with dual feedback architecture shows robustness in its performance [6].

At last, this study concludes that the use of dual feedback architecture design could ensure the robustness of the system's performance. For future work, the dual feedback architecture could be used to design synthetic oscillator with imitation to the natural biological system in order to achieve stable and robust oscillation.

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