Building Reliable Genetic Devices using Unreliable Ones

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1 Overview

Synthetic biology is an engineering discipline that attempts to create devices that do not normally exist in nature [1, 2, 3]. Standardization of genetic parts, devices and systems, known as *Biobricks* [3, 4, 5], has made a considerable impact on the research of engineered circuits that work within the cell, resulting in applications such as smarter drugs that respond to external conditions adaptively, biofactories that produce cheaper organic materials and alternative cancer treatments [2, 6, 7, 8]. Among the many design methodologies for genetic circuits, a very popular one uses analogy with electrical circuits, particularly digital ones, which are easy to design, robust to cumulative errors and noise.

As in the case of electrical devices, genetic ones are not fully reliable and may fail in a stochastic fashion. For example, the experimental studies reported in [9, 10] show that for a large number of genetic switches working in parallel, the output exhibits a bimodal distribution due to cell-to-cell variations and the stochastic nature of biological systems. This effect can accumulate in larger networks, and can be detrimental in applications such as drugs and cancer treatments [9, 7] where being able to turn on or off a given set of genes accurately with respect to the concentration of single or multiple biochemical factors is crucial.

In this project, we propose to investigate novel ways of building reliable genetic devices. Consider for example the problem of detecting the presence of some combination of manmade toxic chemicals. Suppose we would like to determine whether proteins A and B are present together or proteins A and C are present together. Using the Biobricks setup, we can create *NOT* and *NOR* logic gates [11] whose promoters may be affected the presence of these proteins. Such a circuit might be expressed algebraically as NOR(NOT(A), NOR(B, C)), where the final NOR is attached to a green fluorescent protein (GFP), whose presence indicates an affirmative answer to the original question. Achieving greater reliability would ensure that the GFP would appear if and only if the proper combination of inputs appeared. More ambitious would be to deliver drugs according to a schedule if this triggering event occurred. To achieve time release, we would use a clock design based on a so-called repressilator [**?**]. At each clock cycle a certain amount of drug would be delivered.

One of the simplest components of the above system is a *genetic switch*, which takes the output of the final NOR gate, and results in a desired level of GFP to trigger the clock. A genetic switch in general is a transcriptional network where the input is the concentration of a transcription factor or a regulator (for example a repressor or an activator), and the output has two distinct levels of some other protein concentration, defined as *ON* or *OFF* states [3]. One of the best-known genetic switches is the λ -Phage switch [12], which manages a phage to enter into the lytic or the lysogenic cycles. The exact mechanism how this switch works is well-understood [12, 13]. Multimerization of the transcription factors and colloborative binding result in more reliable switches, by simply binding two repressors whose effect is larger than the sum of individual effects [1, 12, 13]. The efficiency of such systems is shown in Figure 1, by comparing this switch with a single repressor-operator system.

Starting with this simplest component, how can we ensure reliability? Most research on the reliability of genetic switches either re-engineers proteins and other regulatory elements or manipulates the network architecture among simple genetic parts [3, 9]. Our proposed approach, on the other hand, is to *design more reliable genetic switches by building networks of unreliable ones.* Motivated

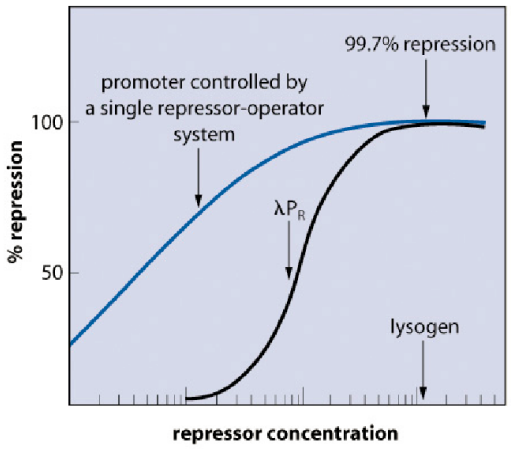

Figure 1: *Repression as a function of repressor concentration in two systems [12]. The black line represents a more reliable (steeper) system.*

by the analogy with electrical circuits, we will investigate the approach of Shannon [14] where he proved that *arbitrarily* reliable relay[1] operation can be accomplished by proper interconnection of a sufficient number of unreliable relays. We first briefly describe Shannon's framework before summarizing our proposed work.

2 Relay Networks

Shannon considered the scenario in which one has access to a large number of unreliable relays, and needs to build a single reliable one [14]. To model a relay, he viewed it as a channel with binary input X, and binary output, Y. He also defined the transition probabilities between the input and outputs as; $P(Y=1|X=1)=\alpha$, $P(Y=1|X=0)=\beta$ as shown in Figure 2. For a reliable relay, we need α to be close to 1, and β to be close to 0. Shannon defined the reliability function $h(p)$ as the probability that the output is 1 for a network of relays, where $p=P(Y=1)$ for an individual relay. Note that with this definition, a network serves as a more reliable relay than the original one if $h(p)\leq p$ for $p\leq p^*$ and $h(p)\geq p$ for $p\geq p^*$ for some $p^*\in(\beta,\alpha)$ with $h(p^*)=p^*$, simply indicating that by forming the network, the effective α has increased, while the effective β has decreased. This is illustrated in Figure 2. Using this approach, Shannon analyzed the interconnection of different networks (or network of networks) and proposed a design methodology leading to an arbitrarily reliable relay given enough number of unreliable relays.

3 Proposed Work

We first describe our proposed work within the context of a genetic switch, then discuss extensions to more complex components and devices. Our goal is to study whether a Shannon-type approach is applicable to building reliable genetic devices. We start with a mathematical formulation which will then prescribe practical implementation methodologies.

The first step is to mathematically model both the genetic switch and the interaction of multiple switches. Specifically one would need to obtain a "calculus" of genetic switches that describes the

[1] A type of electrical switch.

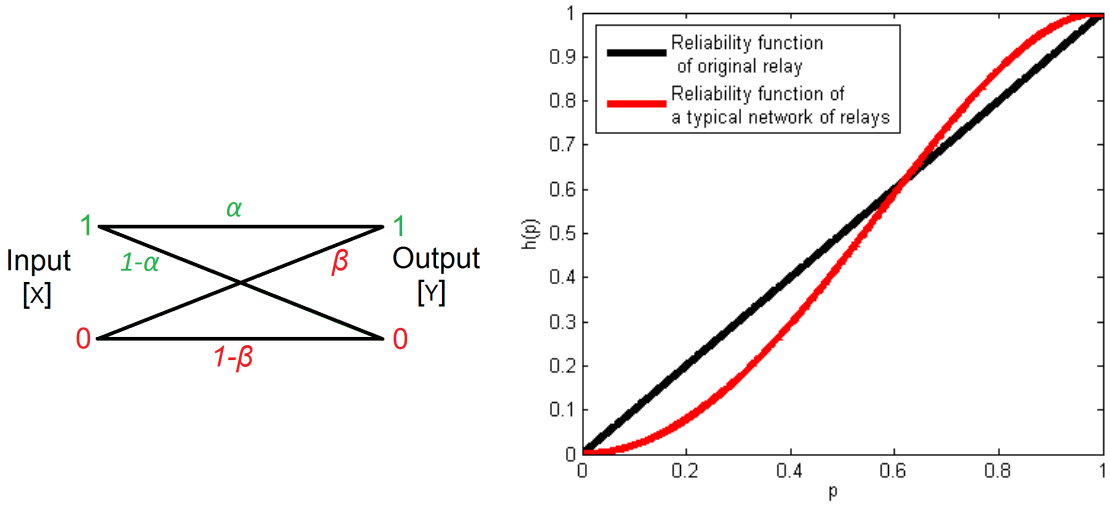

Figure 2: *Left: Representation of a relay. Right: Typical reliability functions.*

input-output relationships of various switch connections as a function of the individual switch model and the type of connection, as done by Shannon for relays, where three types of connections, *series*, *parallel*, and *composite*, were considered [15]. Once such models are obtained, the next step would be to modify Shannon's analysis for transcriptional switch networks and investigate general properties of such networks that form an effective reliable switch. An important question would be how physical restrictions on the size of the network would affect the overall switch reliability.

To illustrate the feasibility of such an approach, we present a very simple probabilistic model of a genetic switch. The input to the switch is taken as the concentration of an activator, the output of the switch is the concentration of another protein, for example a GFP as above. We let α to be the probability that an output molecule is produced while the input concentration is *high*, and β be the probability that an output molecule is produced when the input concentration is *low*. While this approach is promising, it has several shortcomings that need to be addressed for a more accurate model. Assuming constant α and β ignores self regulation of the genes and modeling transcription/translation chain with a binary channel may be an oversimplification. Furthermore, genetic switches do not work independently, unless the network is small [16].

Our proposed work includes incorporating the above issues into our model and design. Our theoretical investigations will go hand in hand with the simulation approach, which is facilitated by the availability of genetic simulation softwares (see [2] for a list of simulators). This will be followed by an implementation of the engineered genetic networks using parts from MIT Standard Registy [11]. For the implementation stage we plan to establish cooperation with a molecular biology lab. Finally, we plan to extend the above network approach to build other reliable genetic devices such as oscillators, counters and detectors [2, 3], for which we will need to address the concepts of delay and feedback and include cyclic connections.

We expect that our approach, if successful, will provide a new paradigm for building genetic devices and circuits by making use of unreliable components, which can be manufactured easily in abundance. It will also allow a new direction for information theory research (a research area that was started by Shannon), reinforcing recent emphasis on biological applications of information

theory [19].

4 Project Team and Plans for External Support

The project team consists of Elza Erkip (NYU-Poly), Dennis Shasha (NYU, Courant), and their two students who will be supported by this grant. Elza Erkip is an expert in information theory; she has won several awards for her work in network information theory and wireless communications. Dennis Shasha (Dennis: please add one sentence here).

During the first year of the project, we plan to seek for external funding through NSF. This seed grant will allow us to devote time and resources (specifically student funding) to obtain an initial set of results. Note that this is a completely new direction of research, so we will need to show some initial success to convince the grant agencies. We expect to complete preliminary theoretical work within six months to submit a full proposal to NSF's Communication and Information Foundations (CIF) program which funds fundamental research in information theory (Deadline December 17, 2013). Specifically we will start with simple abstract models and illustrate feasibility by obtaining some theoretical results and by providing an initial set of simulations. The next six months of seed funding will allow us to refine the models and the analysis, and (ELZA MORE) until external funding is obtained. Given the excitement in the information theory community in investigating biological applications [19], we expect significant interest from NSF's CIF program. (MORE on FUTURE FUNDING).

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