Practical Properties of Biological Switches

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***Short Abstract* — As synthetic biology moves from technology development to practical use, which circuit to choose for a particular function becomes a question of some importance. As part of a project that is constructing synthetic switches in plants, we require switch designs that are bistable, and can deliver the required performance, such as control of unavoidable leaky expression. This work asks the question: which circuit topology is best for controlling these practical properties. We study the characteristics of two classic circuit designs using deterministic and stochastic modeling. This mathematical analysis is based on a suite of quantitatively characterized promoters in Arabidopsis experiments.**

I. Introduction

Many circuit topologies can be used to make a biological switch [1,2,3]; however, their deterministic and stochastic properties are quite different. The two most common circuit topologies are based on either positive feedback, or mutual repression, and have been extensively characterized previously for properties such as control of noise and, for the mutual repression circuit, stochastic switching between states [4,5,6]. However while constructing biological switches for practical synthetic biology applications, we were faced with other practical questions such as (i) which type of switch yields the maximum fold change; (ii) which type of switch has the most tightly controlled OFF state, given that promoters are leaky; (iii) which type of switch remains bistable under greater parameter variations etc. In particular, real promoters are leaky, and the robustness of biological switches against leaky promoters, rather than intrinsic or extrinsic noise becomes a matter of importance. Characterizing these practical design properties will expand the knowledge in the field, and facilitate the use of the appropriate switches in practical applications.

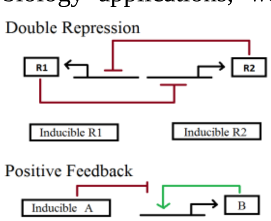

Figure 1
The two circuit topologies.

In this study we first do experiments on synthetic plant parts to characterize them mathematically. Then we use deterministic and stochastic simulations of circuits made of these experimentally characterized parts in plants to study these questions.

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II. Method

To compare the mutual repression circuit with positive feedback circuit described in figure 1, we looked at four properties: 1. The size of the region of bistability in parameter space for the two systems using parameters derived from experimental studies of the genetic components in plants. 2. The effect of leaky expression of the promoters on the size of this parameter space. 3. The quasipotential landscapes of the switches in the low molecule limit and the effect of leaky expression on the quasipotential. 4. The rate of stochastic switching between states.

Parameters were estimated experimentally as follows. For the mutual repression circuit Arabidopsis protoplasts were transformed with synthetic constructs containing a luciferase reporter under the control of a repressor which itself was under the control of an inducible promoter. Florescence data was collected for these individual promoter-repressor pairs, and was then fit to hill functions. For the positive feedback circuit, parameters were chosen that could reproduce the results seen in Arabidopsis whole plant studies and then modified to estimate what the parameters would be if the plants were in the bistable region.

We used bifurcation analysis of the circuits to make phase diagrams to compare the size of the bistable region. Further, we used stochastic simulations to study the quasipotential landscape of the circuits. Data analysis is still underway, but we present our preliminary results below.

III. Conclusion

We found that leaky expression of the inducible promoters surprisingly increase the bistable region for the positive feedback switch and decrease it for the mutual repression switch. We also found that the robustness of the ON and OFF states increased faster for the positive feedback circuit as molecule number increased. Altogether our preliminary results suggest that the positive feedback based circuit is better at handling the leaky expression from the inducible promoter as well as maintaining a high fold change with low concentrations of other key proteins in the system.

References

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