

Modeling Multiple Steady States in Genetic Regulatory Networks

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From networks of simple regulatory elements, scientists have shown some simple circuits such as the circadian oscillator¹ or the toggle switch². These circuits utilized the multistability inherited in the regulatory elements. The multistability in genetic regulatory networks inspired our group to experiment modeling multiple steady states. Our motivation is to eventually use these steady states to store information in a reliable way. Ideally, a transient input would lead to a permanent storage unit. We can also create multiple steady states to implement the finite state machine. Furthermore, relevant applications in biocomputing and gene therapy³⁻⁴ can benefit from this research. Using four repressible promoters arranged in a mutually inhibitory network, four steady states can be realized. We use transient chemical or

thermal induction to go from state to state.

The toggle switch experiment exhibited a nearly ideal switching threshold; hence, with the right inducers switching threshold in a four steady states system would not be a problem.

What is a steady state?

In the context of genetic regulatory networks, a steady state⁵⁻⁶ is a condition where there is no net change in the system.

The system is stable if slight perturbations bring the system back to its steady state.

More significant perturbations may move the system to a new steady state.

The four steady states system in this case is composed of four promoters and four corresponding repressors (Fig. 1). A promoter transcribes its repressor, which, in turn, inhibits the other three promoters.

There are four possible stable states:
promoter A transcribes repressor A,

promoter B transcribes repressor B,
 promoter C transcribes repressor C, and
 promoter D transcribes repressor D. By
 transiently introducing an inducer, a
 repressor is maximally transcribed until it
 stably represses the other three promoters.

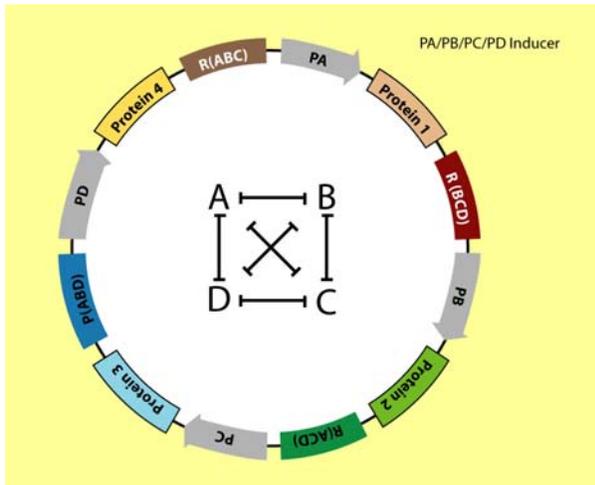


Figure 1 Four steady states system.

Since the proposed four steady states system is a direct extension from the toggle switch experiment, we varied the steady states equations found in Gardner's research to correspond to our proposal. We can mathematically visualize the behavior of the system by understanding the following equations modeling for the network:

$$\frac{dA}{dt} = \frac{\alpha_A}{1+B^{\beta_b}} + \frac{\alpha_A}{1+D^{\beta_d}} + \frac{\alpha_A}{1+C^{\beta_c}} - A$$

$$\frac{dB}{dt} = \frac{\alpha_B}{1+A^{\beta_a}} + \frac{\alpha_B}{1+D^{\beta_d}} + \frac{\alpha_B}{1+C^{\beta_c}} - B$$

$$\frac{dC}{dt} = \frac{\alpha_C}{1+D^{\beta_d}} + \frac{\alpha_C}{1+A^{\beta_a}} + \frac{\alpha_C}{1+B^{\beta_b}} - C$$

$$\frac{dD}{dt} = \frac{\alpha_D}{1+C^{\beta_c}} + \frac{\alpha_D}{1+A^{\beta_a}} + \frac{\alpha_D}{1+B^{\beta_b}} - D$$

Where A is the concentration of repressor A, B is the concentration of repressor B, C is the concentration of repressor C, D is the concentration of repressor D, α_A is the effective rate of synthesis of repressor A which represses promoter B, C, and D, β_A is the cooperativity of repression of promoter A, etc... The terms with α and β in every equation are the cooperative repression of constitutively transcribed promoters, and the last term in every equations is the degradation of the repressors.

The parameters $\alpha_A, \alpha_B, \alpha_C, \alpha_D$ factor into the equation the net effect of RNA polymerase binding, open-complex formation, transcript elongation, transcript

termination, repressor binding, ribosome binding and polypeptide elongation. The parameters $\beta_A, \beta_B, \beta_C, \beta_D$ come from the multimerization of the repressor proteins and the cooperative binding of repressor multimers to multiple operator sites in the promoter.

To reach steady states $\frac{dA}{dt}, \frac{dB}{dt}, \frac{dC}{dt}, \frac{dD}{dt}$ must

approach zero. $\frac{dA}{dt}$ means change in concentration of the repressor A over time.

As $\frac{dA}{dt}$ approaches zero, there is no net change, hence multistability occurs. Using the steady states equations aforementioned, we utilize available mathematical software such as Mathematica or Matlab to solve for the range of the effective rate of synthesis for the repressors and the range of the cooperativity of repression of the promoters. Once we've found the ranges for these unknown variables, we can find repressors and promoters of which the effective rate of synthesis for the repressors and the

cooperativity of repression of the promoters fit into these ranges. We would test these repressors and promoters in vitro to see if they actually fit into our model.

As simple as it might sound, there are problems that we suspect might surface along the way. First of all, the steady state equations are quite complex. For a four steady states system, there are eight unknown variables in four coupled differential equations. This can be solved when we take into account the symmetry of the equations. We are positive that Matlab can crunch out the numbers. Given that we can find the numbers for α and β , it is biologically infeasible. We are not sure if we can find promoters and repressors that fit into the ranges specified by the equations. Finally, four rates have to be considered simultaneously. Since one of the applications of the multiple steady states system is implementing the finite state machine⁷, there will be timing issue. The

rates will vary, meaning transferring from state to state requires different amount of time which is a set back since state transition is based on clock edge trigger in the current technology. Information storing can be complicated when accessing one unit takes longer time than another. The problem does not stop here. The complexity will scale up exponentially when the number of states increases. After realizing such problems, we proposed another method to reduce the complexity. We decided to split the system into two parts. The new system is strikingly similar to the toggle switch model. Instead of one pair of promoters and repressors on the plasmid, we have two pairs interacting independently of each other. Since the toggle switch model has been shown as a working model, the newly proposed model is also very likely to work. If we want to achieve more states, this model is still a bad design.

We were hoping to test out some of our models to see what the result will be. However, given the time we had, it is impossible to find a lab and equipments to carry out such an elaborated experiment. As for now, we are still looking for other means to implement what we set out to do. We also are trying to come up with a more efficient design to realize the multiple steady states without complications exponentially scale up as the number of states increases.

Reference:

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