

## Modeling and Design of Gene Regulatory Modules: From Networks to Molecules

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Armed with increasingly fast supercomputers and greater knowledge of the molecular mechanisms of gene expression, it is now practical to numerically simulate complex networks of regulated biological reactions, or gene circuits. Using an exact stochastic simulation algorithm, we obtain an accurate time-evolution of the behavior of complex gene circuits. Specifically, we examine important gene circuit modules, such as the bistable switch, and use the stochastic simulation algorithm to develop design principles that will enable us to produce fast and robust gene network modules for use in genetic engineering applications.

We move from the network to the molecular level, translating the network design principles to requirements for specific binding strength between proteins and DNA molecules. Using atomistic resolution simulations we calculate the binding affinity of regulatory protein-protein and protein-DNA interactions. The high-resolution picture of atomistic simulations enables us to computationally engineer regulatory proteins that facilitate tight control of entire gene networks. We thus have a protocol that allows us to model and design gene regulatory circuits *in silico*, with a top-to-bottom approach.

We have successfully used our method<sup>1</sup> to construct a design for a high-certainty bistable switch genetic circuit, consisting of two genes  $D_1$  and  $D_2$ , each producing a repressor that represses the expression of the other gene. The switch has two significant dimensionless numbers; they are state and certainty. The state of the switch is either “On”, where the number of  $D_2 > D_1$ , or “Off” otherwise. The certainty of the switch determines to what extent the output signal, either on or off, is reliable and accurate, and is described by  $C = |D_2 - D_1| / (D_2 + D_1)$ .

One of the design principles, resulting from the network simulation, is that the certainty of the switch increases by decreasing the repressor-operator dissociation constant (Figure 1).

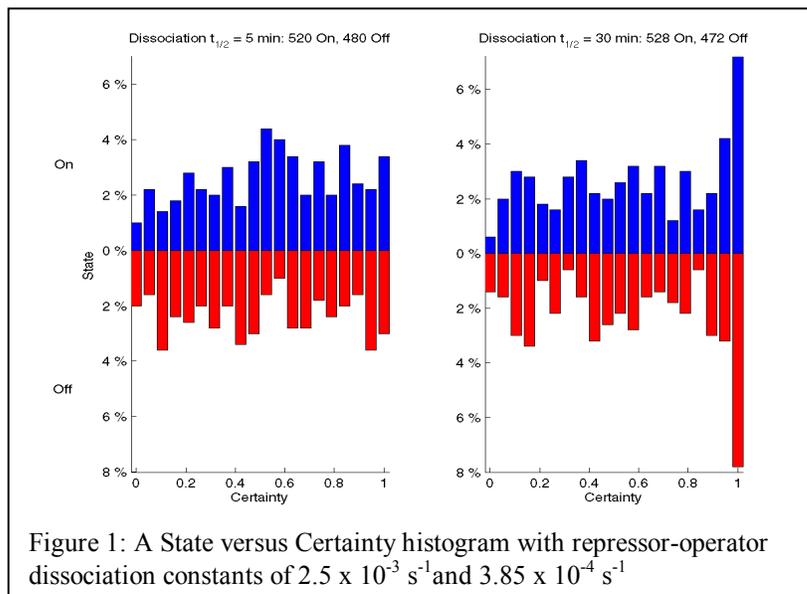


Figure 1: A State versus Certainty histogram with repressor-operator dissociation constants of  $2.5 \times 10^{-3} \text{ s}^{-1}$  and  $3.85 \times 10^{-4} \text{ s}^{-1}$

Dissecting the structures of known repressor-operator complexes we use computationally efficient simulations to calculate the binding affinity of repressor-operator complexes and identify the protein residues that play a central role in binding and are amenable to mutations. We perform computational mutation experiments and calculate the relative binding affinities, developing molecular design principles for a high-certainty bistable switch. In the presented poster we will describe the computational systems biology methods developed in our lab that enhance our understanding of complex, molecular-level biological events and confer the ability to design diagnostic and therapeutic solutions.

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