# Experimental Design of *In Vitro* DNA Reaction Networks

J. Bishop, D. Georgiev, and E. Klavins

***Short Abstract*— We consider the integration of analytical and experimental tools for the design of engineered biochemical networks. We derived an experimental design tool that discriminates candidate models and exploits the disparities to test their viability. We further developed a new experimental testbed that is conducive to model invalidation and efficient, parallel experimentation. The tools will be integrated to reliably model and design various *in vitro* DNA reaction networks.**

## I. Introduction

The design of engineered biological systems is complicated by a general lack of models that predict dynamic behavior. With the exception of certain limited classes (e.g., monotone systems [1]), biochemical reaction network models often fail in prediction due to unknown parameters or wrong abstraction level. Therefore, general, reliable, and predictive mathematical models are needed to effectively engineer biological systems and their component parts (e.g., regulators, bi-stable switches, and oscillators).

We have developed tools for experimental design and execution. The experimental design tool is a general analytical method that discriminates candidate models and exploits any disparities to identify viable parameter regimes and abstraction levels. The experimental execution tool is an experimental testbed for *in vitro* biochemical systems that are mainly built from DNA and RNA. These tools form the foundation of a growing set of tools we are developing for reasoning about and testing models of biochemical reaction systems.

## II. Methods

The experimental design tool takes a pair of candidate models and computes, if possible, experimental conditions that will necessarily indicate which candidate model is viable [2]. First, an experiment's controlled variables (e.g., molecular concentrations) and uncontrolled variables (e.g., reaction rates) are modeled as the control input $u$ and disturbance $w$, respectively. Then, given a pair of candidate input-output models $y_1 = M_1(u, w)$ and $y_2 = M_2(u, w)$, with the same input and output spaces, we solve the following two problems: P1) *Model Discrimination Problem* – find an input, called the *disparity certificate*, that yields different outputs for all possible disturbances; P2) *Model Invalidation Problem* – given the inputs and outputs for a series of executed experiments, find which candidate model maps the inputs to different outputs for all possible disturbances.

J. Bishop, D. Georgiev, and E. Klavins are with the Department of Electrical Engineering, University of Washington, Seattle, WA 98195-2500, USA jdbishop@u.washington.edu, dgeorgie@u.washington.edu, klavins@u.washington.edu

The mappings $M_1$ and $M_2$ are given implicitly by solving the differential equations representing the biochemical reaction networks. The complex relationship between the inputs and the outputs resulting from the typical nonlinearity of these differential equations makes problems P1 and P2 intractable. We derived a sufficient method for solving the problems using a scalable convex relaxation and showed how the disparity certificate generates experimental data that necessarily invalidates at least one of the two candidate models.

The experimental tool is a testbed for testing models of biochemical reaction networks on *in vitro* DNA and RNA-based dynamical systems. For example, we have used experimental data from the testbed to compare a pair of competing biochemical reaction models of a DNAzyme-based, RNA-fuelled nanomotor [3]. The favorable comparison of simulation data from one model–and not of the other–to experimental data shows that waste management is crucial to achieving the desired system behavior. Recently, we have begun working with systems built from synthetic transcriptional switches [4] and have also added high-throughput, parallel experiment capabilities to the testbed.

The experimental testbed could be used to directly search for controlled variables that yield specific outputs. Such a search, however, may be overly time-consuming and may yield little predictive power. Instead, we consider using disparity certificates to reduce a set of candidate models in an exponential fashion.

## III. Future Work

We will fully integrate the above experimental and analytical tools to conduct a parallel search for a mathematical model that best predicts the experimental output. The basic approach comprises the following steps: 1) formulate candidate models by partitioning the parameter space, and 2) discriminate among the candidates to refine the viable set of parameters. The above two steps are repeated until disparate models can no longer be found. Numerical and experimental implementations of these steps are currently being developed.

## References

[1] E. D. Sontag, "Monotone and near-monotone biochemical networks," *Systems and Synthetic Biology*, vol. 1, pp. 59–87, 2007.

[2] D. Georgiev and E. Klavins, "Model discrimination of polynomial systems via stochastic inputs." *submitted to Proc. IEEE Conf. Decision & Control*, 2008.

[3] J. Bishop and E. Klavins, "An improved autonomous dna nanomotor," *Nano Letters*, vol. 7, no. 9, 2007.

[4] J. Kim, K. White, and E. Winfree, "Construction of an in vitro bistable circuit from synthetic transcriptional switches," *Molecular Systems Biology*, vol. 2, no. 68, 2006.