Building the Components for a Biomolecular Computer

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Abstract. We propose a new method for amorphous bio-compatible computing using deoxyribozyme logic gates [1] in which oligonucleotides act as enzymes on other oligonucleotides, yielding oligonucleotide products. Moreover, these reactions can be controlled by inputs that are also oligonucleotides. We interpret these reactions as logic gates, and the concentrations of chemical species as signals. Since these reactions are *homogeneous*, i.e., they use oligonucleotides as both inputs and outputs, we can compose them to construct complex logic circuits. Thus, our system for chemical computation offers functionality similar to conventional electronic circuits with the potential for deployment inside of living cells. Previously, this technology was demonstrated in closed-system batch reactions, which limited its computational ability to simple feed-forward circuits. In this work, we go beyond closed systems, and show how to use thermodynamically open reactors to build biomolecular circuits with feedback. The behavior of an open chemical system is determined both by its chemical reaction network and by the influx and efflux of chemical species. This motivates a change in design process from that used with closed systems. Rather than focusing solely on the stoichiometry of the chemical reactions, we must carefully examine their kinetics. Systems of differential equations and the theory of dynamical systems become the appropriate tools for designing and analyzing such systems. Using these tools, we present an *inverter*. Next, by introducing feedback into the reaction network, we construct devices with a sense of state. We show how a combination of analytical approximation techniques and numerical methods allows us to tune the dynamics of these systems. We demonstrate a flip-flop which exhibits behavior similar to the RS flip-flop of electronic computation. It has two states in which the concentration of one oligonucleotide is high and the other is low or vice versa. We describe how to control the state of the flip-flop by varying the concentration of the substrates. Moreover, there are large regions of parameter space in which this behavior is robust, and we show how to tune the influx rates as a function of the chemical reaction rates in a way that ensures bistability.

1 Introduction

We use deoxyribozymes (nucleic acid enzymes) as gates to transform input and substrate signals (molecular concentrations) into product signals and thereby perform sim-

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ple computation. Since the inputs are of the same type as the outputs, viz. oligonucleotides, gates may, in principle, be connected in complex circuits, with the output of one gate acting as the input of another. Thus, we may design chemical systems that perform complex computations from simple boolean primitives in much the same way electronic computers are built from simple logic gates. These devices could operate without macroscopic intervention in a biological environment, and the goal of this technology is autonomous *in vivo* computation for diagnostic and therapeutic purposes. We have reported gates with a single layer of logic, and no inter-gate communication [1]. Devices that function as a half-adder [2] and a tic-tac-toe automaton [3] have been built and tested in the laboratory.

These gates have been deployed in a closed reactor, which effectively limits this technology to one-shot boolean computations. To overcome this limitation, we explore using this chemistry in an open reactor, in which gates could be re-used many times and connected in recurrent, rather than feed-forward, circuits. This adds a level of complexity to the engineering task, but we develop a process that may be used to engineer these devices. We apply methods of dynamical systems to construct reaction networks in open reactors that implement rudimentary elements of digital chemical computation. This allows us to investigate complex reaction networks that make use of inter-gate communication and feedback.

2 The Chemical Kinetics of Deoxyribozyme Logic Gates

The four components of our deoxyribozyme system are inputs, gates, substrates, and products. Under certain input conditions a gate is an active enzyme [1]. The effect of input molecules on the catalytic activity of the gate defines the logic operation that the gate performs. A gate requires the presence and/or absence of certain inputs to be active. When active, the enzymatic gate is a phosphodiesterase: it catalyzes an oligonucleotide cleavage reaction. A substrate molecule is cleaved into two product molecules. The product molecules represent the output signal of the gate. Computations are carried out in solution, where gates communicate by diffusion of oligonucleotides. Logic signals, true or false, are expressed by high or low concentrations of specific oligonucleotides. Oligonucleotides transmit information by participating in the reactions of multiple gates. The simplest example is an oligonucleotide that is a product of one gate and an input to another; serving as a substrate would suffice as well.

The mechanism of a deoxyribozyme gate is as follows. Input molecules bind to the designated locations on the gate molecules. The binding of an input to a gate affects the conformation of the gate, which in turn affects catalytic activity. Under appropriate circumstances, the gate is an active enzyme, in which case it binds to a substrate molecule, cleaves it into two molecules of product, and separates into two molecules of product and one active gate complex. Active gates continue to operate as long as there is substrate remaining to be cleaved.

In order to design larger circuits, we must first understand the dynamic behavior of individual logic gates. We set up the experiment as follows. We prepare a solution with a concentration of $G = 250\,\text{nM}$ of a specific YES gate (which becomes active in the presence of input), a certain concentration I of the matching input, and a