Bistability and oscillators in molecular networks built with RNA aptamers

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Bistable circuits and oscillators are essential building blocks in all computing devices, as they enable memory storage and synchronized behaviors. There is evidence that biological cells also heavily relies on bistable systems and oscillators for similar purposes. For instance, bistable gene networks trigger developmental pathways and underlie intra-cellular signal transduction [2]; circadian rhythms time entire organisms, and local clocks govern single cell growth and division [1]. A useful approach to identify the design principles underlying these ubiquitous circuits is to rationally build their minimal versions from the bottom–up *in vitro*, where we can achieve a greater control over experimental parameters [3, 4, 5]. This approach can also yield novel molecular machines, which may be useful as timing devices or pattern generators in bio- and nanotechnology. In this poster we describe the construction of aptamer-based molecular networks with the capacity for oscillations and bistability. Aptamers are an easily expandable tool for *in vitro* and *in vivo* synthetic biology, because aptamers binding arbitrary organic and inorganic targets can be identified with established *in vitro* selection methods [6]. We present mathematical and numerical analysis that demonstrate the feasibility of our designs, and show preliminary experimental results. Preliminary work on these circuits was presented at the 2014 BIOMOD competition [7].

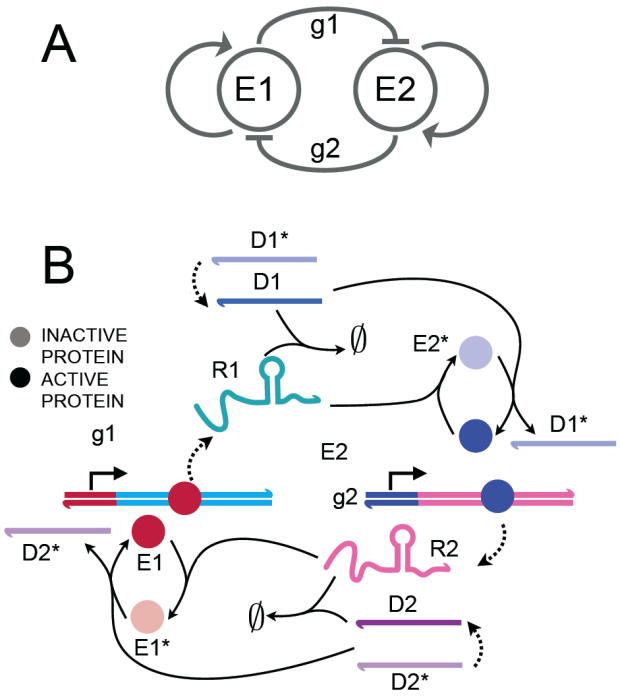

Figure 1: *A: Graph of our aptamer-based bistable switch; E1 and E2 indicate enzymes, g1 and g2 indicate genes. B: Detailed reaction network for the bistable switch. R1 and R2 are RNA species; D1 and D2 are DNA "kleptamers" which activate the ezymes by displacing aptamers R1 and R2. A * indicates inactive complexes.*

The feedback loops necessary to create oscillations or bistability are achieved through regulatory motifs that target directly the activity of two distinct RNA polymerases using RNA aptamers. Recently published aptamers for T7 and SP6 bacteriophage RNA polymerases allow us to build pathways to inhibit or activate production of RNA [8, 9]. These pathways can be reversed by introducing strand displacement reactions, where aptamers are removed from

their binding target by complementary strands we named “kleptamers”. A bistable circuit, for instance, can be built as in Fig. 1 A and B. Two genes produce RNA aptamers R1 and R2, which respectively inhibit enzymes E2 and E1. These inhibition reactions create an overall positive loop (Fig. 1 A). DNA kleptamers D1 and D2 bind to R1 and R2 and displace them from the inactive enzymes $E2^*$ and $E1^*$, thus recovering the ability to transcribe R1 and R2. The displacement of R1 and R2 can be achieved by designing them to have exposed toeholds that do not alter the aptamer core domains. Similarly, we can build an oscillator by adding a species that constitutively inhibits one of the two enzymes, while the RNA produced in the feedback loop serves as an activator.

We build detailed ordinary differential equation models for the proposed circuits and show that they have the appropriate structure to operate as desired. These detailed models build on the simplified networks considered in [10, 11]. When linearized around the equilibrium, the two systems are the positive or negative feedback interconnection of monotone modules [2]. We show that these circuits may undergo exclusively real (positive feedback circuit) or exclusively oscillatory (negative feedback circuit) transitions to instability [12]. These are remarkable properties which hold true for any choice of parameter values (binding rates and total concentrations of enzymes). We conclude that the circuits we designed are structurally well suited for the desired dynamical behaviors. Numerical analysis, where we test behavior of the two circuits in a range of parameters, validates our analytical findings. We report initial experiments characterizing aptamer-mediated enzyme inhibition and reactivation.

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