Load Capacity Improvements in Transcriptional Systems Using Discrete-Time L1-Adaptive Control

[Extended Abstract]

Hamidreza Jafarnejadsani
University of Illinois Urbana Champaign
Urbana, IL 61801, USA
hamid.jafarnejad@gmail.com

Jongmin Kim
Wyss Institute
Harvard University
Cambridge, MA 02115
Jongmin.Kim@wyss.harvard.edu

Naira Hovakimyan
University of Illinois Urbana Champaign
Urbana, IL 61801, USA
nhovakim@illinois.edu

Vishwesh V. Kulkarni*
University of Warwick
School of Engineering
Coventry, CV4 7AL
V.Kulkarni@warwick.ac.uk

1. INTRODUCTION

DNA-based circuits relying on predictable thermodynamics and kinetics of DNA strand interactions impart flexibility in synthesizing synthetic biological constructs and in coupling these circuits to *in vivo* processes [1, 2, 6, 7]. Here, we focus on the synthetic Kim-Winfree oscillator network, illustrated in Fig. 1(i), which is a simple but effective coupled oscillator system in which two DNA switches SW1 and SW2 are coupled through activator and inhibitor blocks realized by RNA signals and auxiliary DNA species (see [3]). A typical experimental realization is *closed* in the sense that once the operation starts, we do not either add any chemicals, especially NTP fuel, externally into the wet-lab apparatus or remove any chemicals, especially waste products, from the apparatus. Within the closed system, the oscillations are bound to die out sooner or later — diminishing NTP fuel eventually stops supporting the production of RNA signals and accumulating waste products clog down the toeholds and, as a result, adversely affect the signal propagation. Furthermore, the oxidation effects and the pH variations tend to deactivate the enzymes. Loading poses an additional challenge since it increases the order and the uncertainty of the system — indeed, these oscillators have recently been used in [8] to drive conformational changes of a DNA nanomechanical device called DNA tweezers. As Fig. 1(ii) shows, the oscillator performance degrades sharply under loading. We propose to improve the loading capacity of such transcriptional devices by adopting a partially open architecture and by using a discrete-time *in silico* controller, a block diagram of which is illustrated in Fig. 2, which is to be coupled to the wet-lab apparatus.

A light switching *in silico* controller, implementing a combination of a Kalman filter and a model predictive controller, was recently reported in [10]. In [4], a continuous-time $\mathcal{L}_1$ adaptive controller was proposed to use a DNA/RNA strand based actuation that faciliates a much greater control channel bandwidth than the one provided by a light-based actuation. In the $\mathcal{L}_1$ adaptive control architecture, the estimation loop is decoupled from the control law. This decoupling allows for the use of fast estimation rates, leading to uniform performance bounds and guaranteed robustness in the presence of bounded nonlinearities and system uncertainties. Hence, the closed-loop system converges to a reference system with partial compensation of uncertainties, which is linear, and hence has a scalable, repeatable, and predictable response. Our discrete-time $\mathcal{L}_1$ adaptive controller builds on the theory developed in [5] which ensures stability for any sampling time. This controller is optimized using a numerically efficient convex optimization method and is well suited for many such bioengineering applications since the frequencies of the reference inputs encountered are slow enough, the biological processes evolve slowly enough, and the wet-lab measurements are at discrete time intervals.

2. MAIN RESULTS

We adopt a partially open architecture analogous to a microchemostat (see [9]) so as to inject control inputs without increasing the reaction volume. In this continuous flow reactor, DNA species, enzymes, and NTP fuel flow in at a low rate, while the outflow removes a portion of reaction mixture, keeping the accumulation of waste products in check. We choose the switch outputs of the main reaction chamber to be the state variables that are to track the desired periodic waveforms. The DNA switches are tagged with fluorophores and the auxiliary DNA activators, A1 and A2, are tagged by quenchers such that the binding of an activator to its target switch reduces fluorescence signal. The two switches, SW1 and SW2, have different fluorophores, allowing for a

IWBDA 2016 Newcastle-upon-Tyrne, August 2016

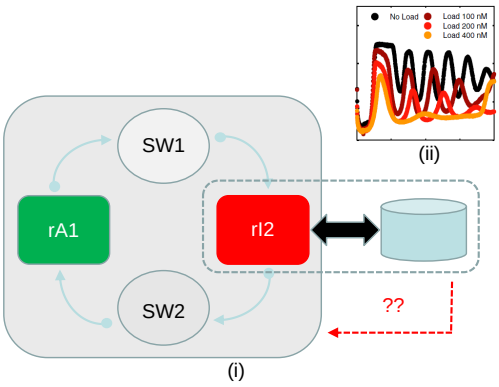

Figure 1: (i) The Kim-Winfree oscillator network comprises two switches (SW1 and SW2) connected through an activator rA_1 and an inhibitor rI_2 block. In [8], it is used to drive DNA tweezers. Due to the loading effects and a closed-system design, this network is unable to drive even moderate loads: the plot (ii), taken from [8], illustrates the loss of oscillations as the load increases from 0 nM to 400 nM. This highlights the need for more sophisticated controllers and for a more open design.

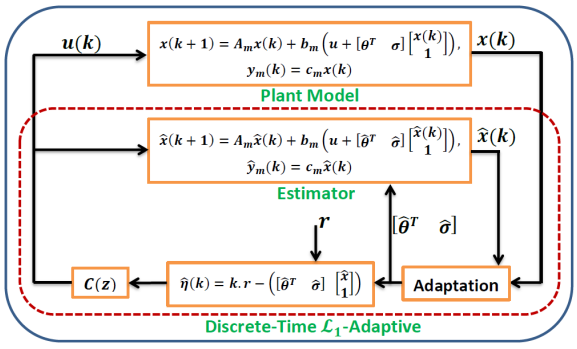

Figure 2: Our discrete-time $\mathcal{L}_1$ adaptive controller. This controller is implemented *in silico* in a computer outside the wet-lab apparatus and the interfaced with the apparatus.

real-time measurement of the switch outputs as fluorescence signals. The target waveforms are generated internally as the reference signals in an *in silico* controller implemented inside a computer — the commands of *in silico* controller controls, in discrete-time, the concentration of inhibitor and activator strands to track the reference signals. We characterize the expected disturbance and modeling uncertainty, obtain a discrete-time model of the overall system to be controlled, and then synthesize a discrete-time $\mathcal{L}_1$ adaptive feedback controller to achieve the desired performance. As Fig. 3 illustrates, a significant improvement in the tunability and loading capacity of oscillator is obtained. This approach can easily be adopted to improve the robustness and the loading capacity of a wide range of wet-lab devices.

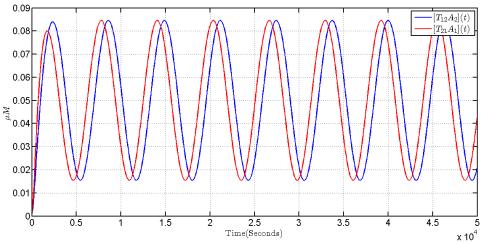

Figure 3: Simulation results for the models of DNA tweezers loaded with 200nM. If the $\mathcal{L}_1$ adaptive controller is not used then the free running system fails to generate any oscillations at all; this has been observed experimentally as well for high load cases (see [8]). However, if our $\mathcal{L}_1$ adaptive controller is used then the desired oscillations are obtained.

3. REFERENCES

[1] Zhang, D.Y. and Seelig, G.: 'Dynamic DNA Nanotechnology using Strand-Displacement Reactions', Nature Chemistry, 2011, 3, (2), pp. 103–113

[2] Padirac, A., Fujii, T., and Rondelez, Y.: 'Nucleic acids for the Rational Design of Reaction Circuits', Current Opinion in Biotechnology, 2012, http://dx.doi.org/10.1016/j.copbio.2012.11.011

[3] Kim, J. and Winfree, E.: 'Synthetic in vitro transcriptional oscillators', Molecular Systems Biology, 2011, 7, 465

[4] Kulkarni, V., Kharisov, E., Hovakimyan, N., and Kim, J.: 'Load Capacity Improvements in Nucleic Acid Based Systems Using Partially Open Feedback Control', ACS Synthetic Biology 3, 617-626.

[5] Jafarnejadsani, H. and Hovakimyan, N.: 'Optimal Filter Design for a Discrete-Time Formulation of L1 Adaptive Control', AIAA InfoTech at Aerospace, Kissimmee, FL, January 2015.

[6] Chen, Y., and Smolke, C.: 'From DNA to Targeted Therapeutics: Bringing Synthetic Biology to the Clinic'. Science Translational Medicine, 2011, 3, 106

[7] Montagne, K., Plasson, R., Sakai, Y., Fujii, T., and Rondelez, Y.: 'Programming an *in vitro* DNA Oscillator using a Molecular Networking Strategy', Molecular Systems Biology, 2011, 7, 466

[8] Franco, E., Friedrichs, E., Kim, J., Jungmann, R., Murray, R., Winfree, E., Simmel, F.: 'Timing Molecular Motion and Production with a Synthetic Transcriptional Clock', PNAS, 2011, 108, E784-E793

[9] Balagaddé, F.K., You, L., Hansen, C.L., Arnold, F.H., Quake, S.R.: 'Long-term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat', Science, 2005, 309, 137-140

[10] Milias-Argeitis, A., Summers, S., Stewart-Ornstein, J., Zuleta, I., Pincus, D., El-Samad, H., Khammash, M., and Lygeros, J.: '*In silico* Feedback for *in vivo* Regulation of a Gene Expression Circuit', Nature Biotechnology, 2011, 29, 12, pp. 1114-1116