Impact of Retroactivity on Transcriptional Components

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***Short Abstract* — Transcriptional components are ubiquitous relays of information in bio-molecular circuits. These components are susceptible to retroactivity, a parasitic effect that can dramatically affect the behavior of a device upon interconnection to a system. In this work we experimentally demonstrate the impact of retroactivity on both dynamic and steady-state response of a transcriptional component. We show that retroactivity increases the apparent Km of the steady state characteristic and introduces a sign-sensitive delay in to input stimulus.**

I. Introduction

Transcriptional regulation is an essential element in the flow of information in bio-molecular circuits. In the field of systems and synthetic biology, these modules are often viewed as information relays whose output is the concentration of a transcription factor [1]. Information coming from this device can be used as an input for other systems through the use of promoters regulated by the said transcription factor. This connection mechanism is susceptible to retroactivity, an effect analog to the concept of output impedance in electrical circuits [2] and previously experimentally demonstrated in signal transduction networks [3]. Yet, retroactivity effects have not been experimentally demonstrated in gene circuits.

II. Results

In this work, we demonstrate through theory and experiments that the impact of retroactivity on a transcriptional component can be dramatic, causing a change of 30% in the apparent Km and an increase of up to 40% in the response time to induction.

Additionally, experiments show that the effect of retroactivity depends on the shape of the input. In particular, retroactivity leads to a slower response time for an induction experiment, but leads to a faster response time for a de-induction experiment. This experimentally observed difference is also of interest as it implies the rejection of one of two competing hypothesis for the behavior of a transcription factor when bound to the promoter. Specifically, studies of retroactivity based on a model in which the transcription factor is stabilized by its binding to the DNA predict a slower response independent of the input shape [2]. However, if the model assumes that the transcription factor is diluted or degraded at a rate independent of its binding state, the sign-sensitive delay is obtained.

The results presented were obtained by fabricating and measuring the response of a transcriptional device, based on the repressor protein LacI, and its corresponding promoter *plac*. In this device, the expression of LacI is regulated by TetR, a repressor protein that, in turn, can be inhibited by addition of anhydrotetracycline (atc), which acts an inducer. The concentration of LacI is measured via the reporter protein GFP regulated by a *plac* promoter. The impact of retroactivity is measured by comparing both dynamic and steady state responses under the presence of additional *plac* binding sites. The mathematical analysis that predicts the behavior observed experimentally is based on singular perturbation theory [4] and orbital equivalence [5].

III. Conclusion

This work demonstrates the difference between the behavior of a transcriptional component when in isolation and when connected in a network. These differences, when assembling or analyzing a circuit, must be taken into account in order to obtain sound predictions about a network behavior. This work also sheds new light on the mathematical models of gene circuits.

References

[1] Alon, U. (2006). *An introduction to Systems Biology: Design Principles of Biological Circuits.* Chapman & Hall/CRC, Boca Raton, FL.

[2] Del Vecchio D, Ninfa AJ, Sontag ED “Modular cell biology: Retroactivity and insulation.” *Mol. Syst. Biol* 4:161 (2008).

[3] P. Jiang et al, “Load-induced modulation of signal transduction networks,” *Science signaling*, vol. 4:194 (2011).

[4] H. K. Khalil, *Nonlinear Systems (3rd Edition)*, 3rd ed. Prentice Hall, 2001.

[5] Y. A. Kuznetsov, *Elements of Applied Bifurcation Theory*. New York, NY: Springer-Verlag, 2004.

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