AFRL-OSR-VA-TR-2015-0080

Quantitative Analysis, Design, And Fabrication Of Biosensing and Bioprocessing Devices in Living Cells

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03/10/2015
Final Report

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AF Office Of Scientific Research (AFOSR)/ RTD
Arlington, Virginia 22203
Air Force Materiel Command

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
02/23/2015	Final Progress Report	06/01/2014 - 05/31/2015

4. TITLE AND SUBTITLE

Quantitative Analysis, Design, And Fabrication Of Biosensing And Bioprocessing Devices In Living Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
FA9550-12-1-0129

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts Institute of Technology -- 77 Massachusetts Ave., Cambridge, MA

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Office of Scientific Research
875 N. Randolph Street Arlington, VA

10. SPONSOR/MONITOR'S ACRONYM(S)

AFOSR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

No restrictions

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of this research is to develop quantitative techniques for the de novo design and fabrication of biosensing devices in living cells. Such devices will be entirely composed of bio-molecular building blocks extracted from living organisms and re-engineered for enhanced functionality. These biosensing devices will be able to reproduce themselves, will be both far smaller and significantly cheaper than current nanoscale electronic devices, and will have the capacity for complex computations.

15. SUBJECT TERMS

phosphorylation, load, synthetic biology, control theory, feedback, dynamics

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		6	Domitilla Del Vecchio
					19b. TELEPHONE NUMBER *(include area code)* (734) 355-8042

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

To: technicalreports@afosr.af.mil

Subject: Final Progress Report to Dr. Hugh DeLong

Grant/Contract Title: QUANTITATIVE ANALYSIS, DESIGN, AND FABRICATION OF BIOSENSING AND BIOPROCESSING DEVICES IN LIVING CELLS
Grant/Contract Number: FA9550-12-1-0129

Period of performance: June 1st 2014-May 31st 2015

PI: Domitilla Del Vecchio

(MIT)

Annual accomplishments

Summary of the Project: This project aims at designing sensing systems in bacteria *E. coli* by employing and re-engineering components from natural sensing/transduction systems. As shown in Figure 1, any such sensing system must have a detector, a transmission system, and a computation element, which produces a visible output. The transmission system usually involves covalent modification cycles such as phosphorylation (the MAPK cascades), while the computation element usually involves gene expression. The properties that we look for in a sensing system are (a) high sensitivity to the presence of molecules to be sensed and (b) fast response time so that the visible output is displayed with minimal delay with respect to when the environmental molecule appeared.

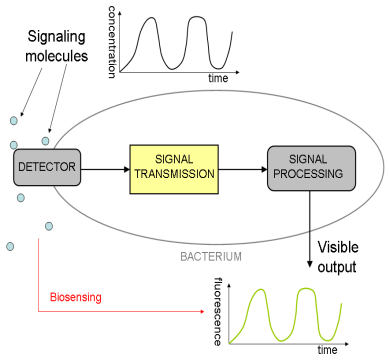

Figure 1: Natural Sensing System. In a cell, the signal transmission machinery is usually performed by networks of covalent modification cycles. The signal processing machinery (computation) is performed by genetic circuits.

Sensing through phosphorylation: Isolation Amplifier Circuit in *E. coli*

The phosphorylation circuit that was assembled in the previous grant to obtain an optimal sensing device is depicted in Figure 2 [1]. This year, we tested the system with its several variants and assessed is temporal response through a new equipment for single-cell real-time measurement.

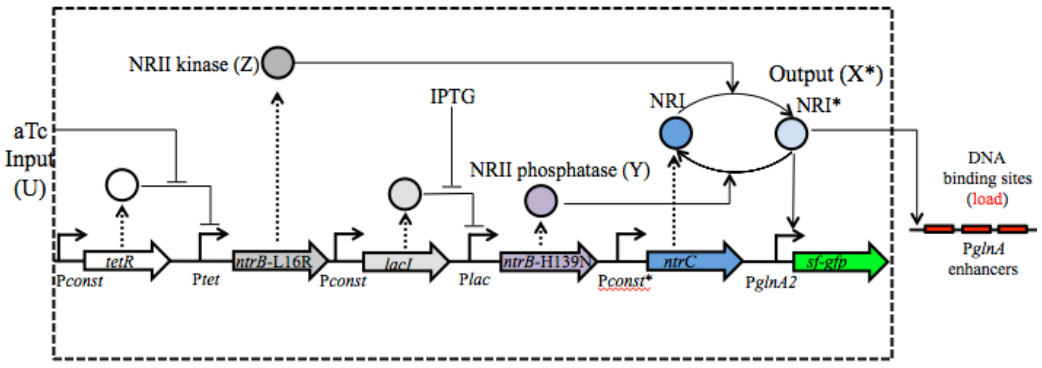

Fig 2. Implementation of a semi-synthetic transmission system based on phosphorylation in *E. coli*. The phosphorylation cycle is given by the NRI-NRI* (phosphorylated) cycle depicted in blue.

Result 1: Making an inverter

We fixed the amount of NRII Kinase by fixing the aTc induction level and then used IPTG as a new input. IPTG induction increases the concentration of the NRII phosphatase and, as a consequence, should decrease the level of NRI* (phosphorylated NRI). The experimental results shown in Figure 3 confirm this prediction showing a linear inverter characteristics.

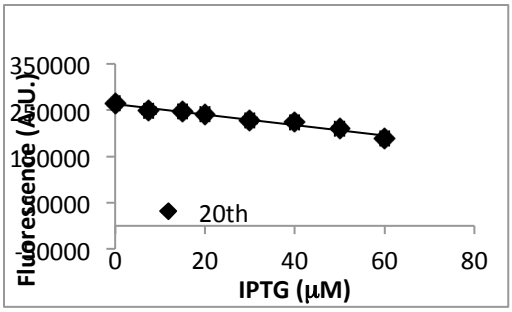

Fig 3. Inverter characteristics of the device.

Result 2: Time-varying induction and automated measurement

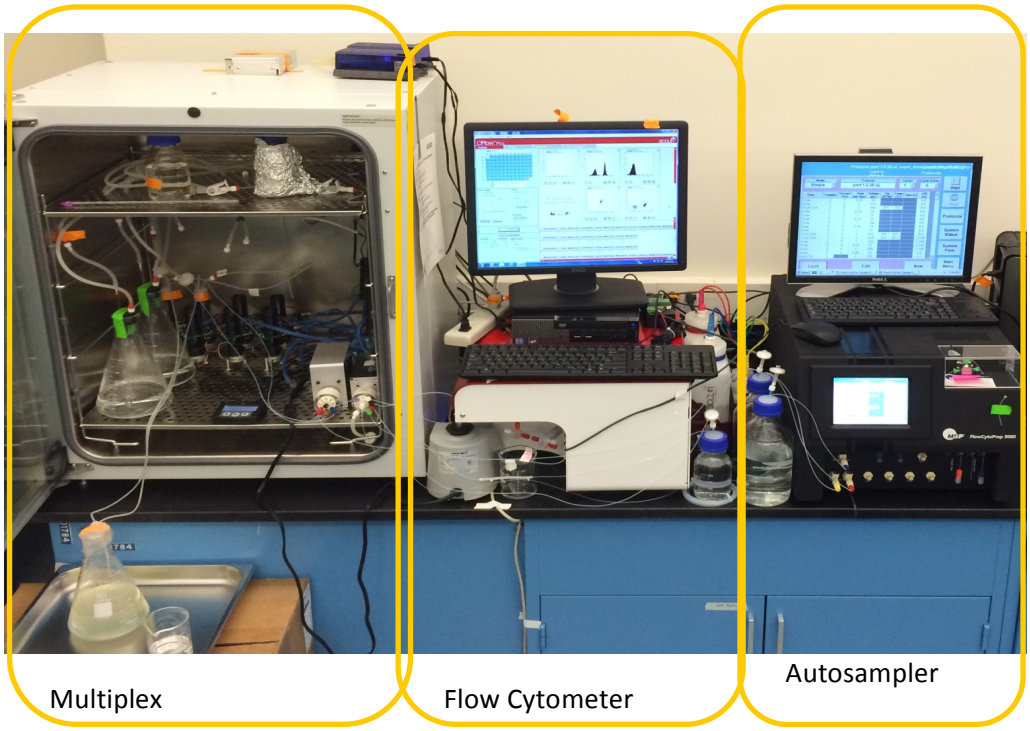

Fig 4. Experimental apparatus for single cell dynamic measurements.

Figure 4 shows the experimental setup if we have assembled to perform time-varying single cell measurements. Specifically, the system is composed of 16 bioreactors (Takahashi *et al*. (2014)), where we have 15 mL per bioreactor at 500 rpm and 30 or 37 ℃. The following customized growth conditions: Batch (maximal growth rate); Turbidostat (constant OD);Chemostat (constant growth rate); Cytostat (constant cell numbers). Also, we have remote control through the wireless network, autosampler with sampling time of 8-10 mins and sampling vol:>25 uL. Figure 5 shows a sample time trajectory where the experiment run for 3 full days. Since the circuit

responds as expected to the time varying induction, it shows that the circuit plasmid is very stable.

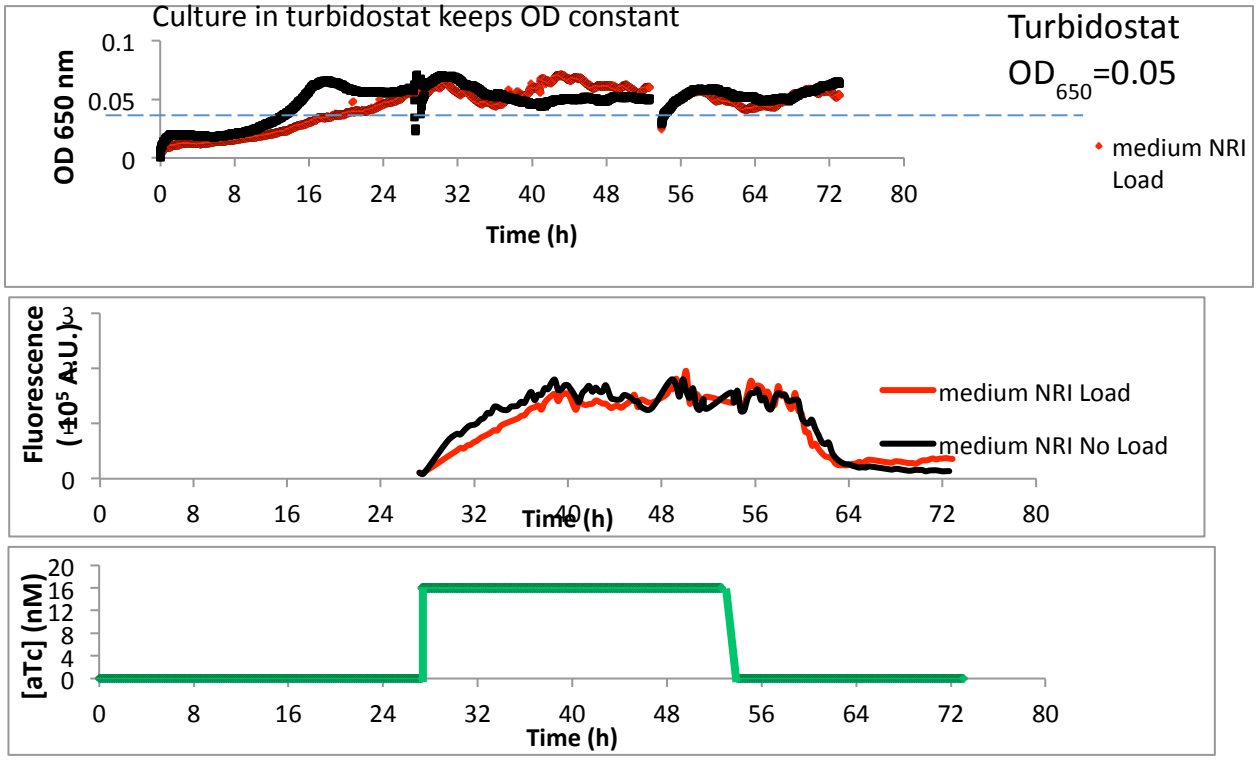

Fig 5. Phosphorylation-based sensing system under time-varying induction..

Result 3: Breaking The Tradeoff with Multiple Stages

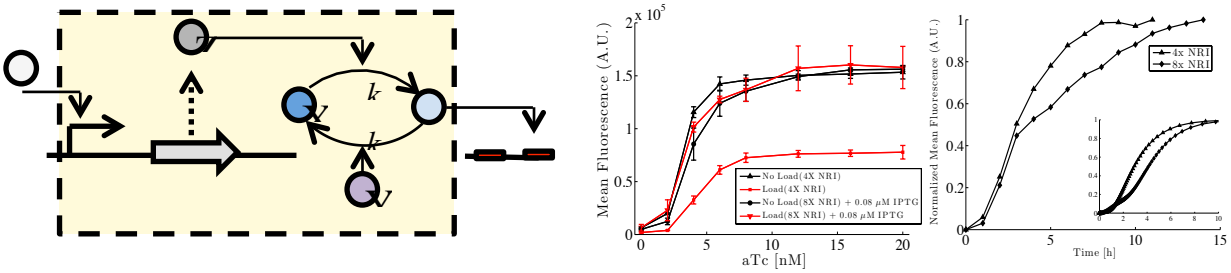

Fig 6. Phosphorylation-based sensing system and its steady state and temporal performance.

Figure 6 shows the steady state and temporal performance of the device based on a single stage phosphorylation cycle. While the system is able to keep a desired input/output characteristic in the face of load, this goes to the expense of a slower temporal response. We were able to overcome this limitation by employing a device based on two stages of phosphorylation.

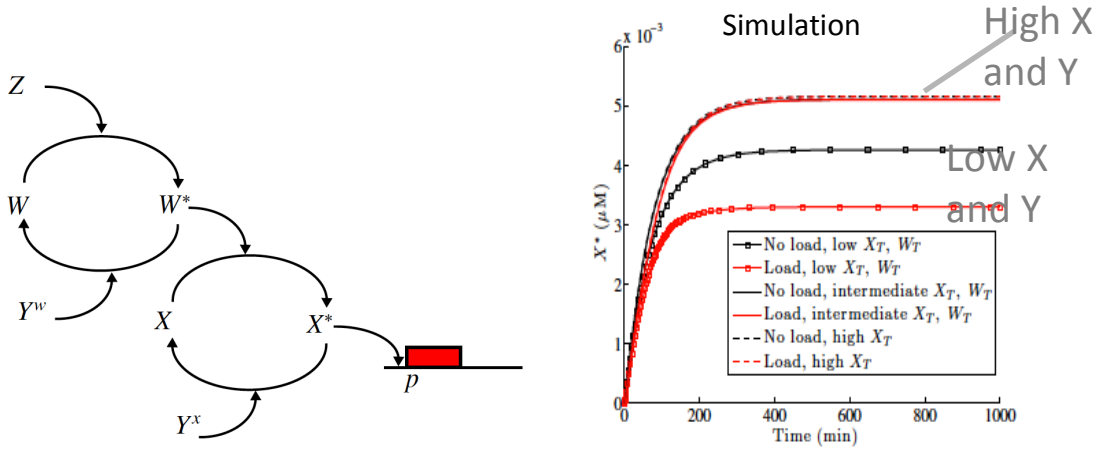

Fig 7. A two-stage device can make the steady state of the output be robust to the load while keeping the desired temporal response.

Figure 7 shows a device with two stages along with simulation results. Because of the presence of the two stages, we were able to use the second stage to attenuate the steady state effect of the load and the first stage fast dynamics to compensate any slow-down in the temporal response due to a high load that X applied on W*. This design was experimentally tested and validated in yeast cells [2].

Noise Properties of the Device

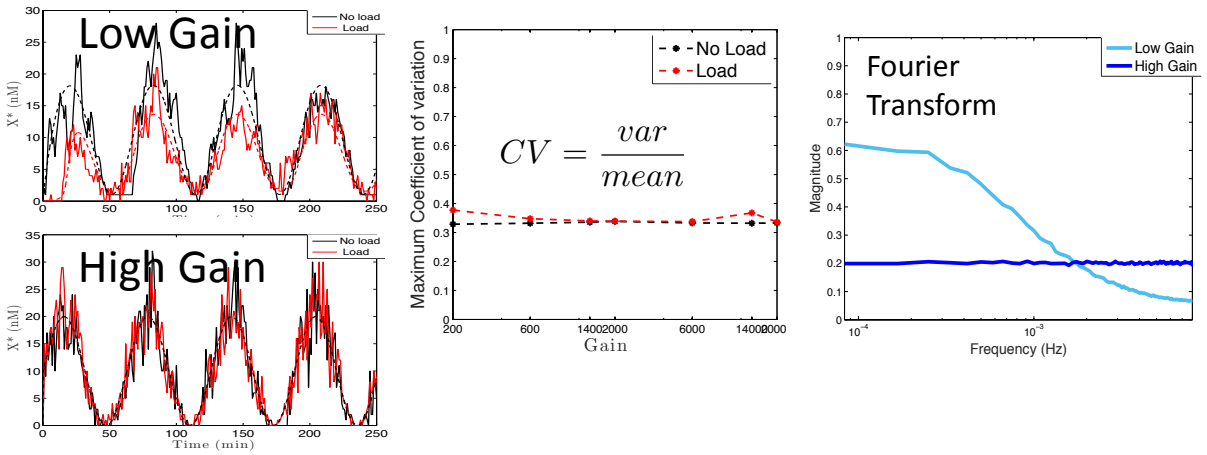

Fig 8. Stochastic simulations performed using the Stochastic Simulation Algorithm (SSA).

Figure 8 shows the noise properties of the device based on a single stage. While the coefficient of variation (CV) is not affected by the gain of the system, the frequency spectrum is. In particular, higher gains tend to decrease the content of the noise of the device at low frequencies but increase it at higher frequencies. Since most useful input stimuli occur on the lower frequency range, this result says that our device possesses also the ability of attenuating noise in addition to attenuating the deleterious effects of the load.

Limitations in Biosensing due to Limitations of Resources

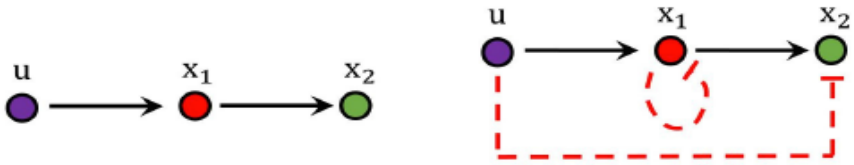

Fig 9. Activation cascade made out of three genes.

Because the transcriptional and translational resources are limited in genetic circuits, hidden interactions arise as multiple genes share these resources. As a consequence a simple activation cascade gives rise (see Figure 9) to hidden interactions (shown in red) that lead to a feedforward loop. Because of this feedforward loop, the ideal input/output response of the cascade (an increasing function) transforms into a biphasic or decreasing one (see Figure 10).

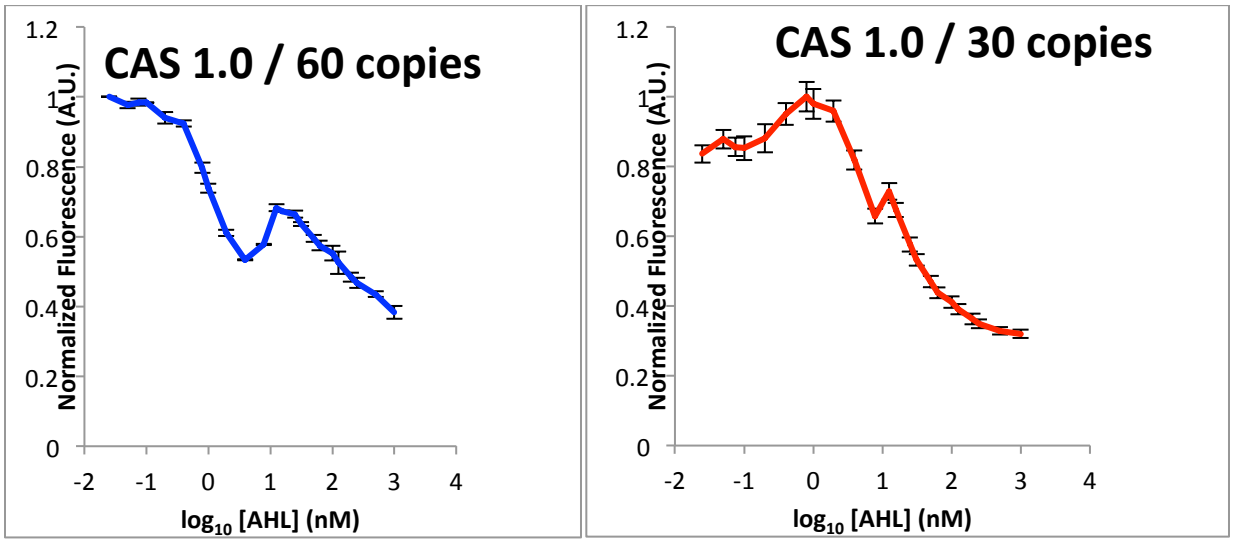

Fig 10. Experimental data showing that, in accordance with mathematical models. The input/output behavior of the cascade can become decreasing of biphasic.

This result shows that the limited availability of resources can cause the sensor to perform in an unexpected and undesirable way. New techniques and research is required to find ways to mitigate the problem and to optimize circuits accordingly.

PAPERS (past year only)

[1] K. S. Nilgiriwala, P. M. Rivera, J. Jimenez, and D. Del Vecchio. Synthetic Tunable Amplifying Buffer Circuit in *E. coli*. ACS Synthetic Biology, October 2014.

[2] D. Mishra, P. M. Rivera, A. Lin, D. Del Vecchio*, and R. Weiss*. A load driver device for engineering modularity in biological networks. *Nat. Biotech,* November 2014.

[3] A. Gyorgy and D. Del Vecchio. Limitations and tradeoffs in gene expression due to competition for shared cellular resources. Proc. of *IEEE CDC*, December 2014

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[5] N. Herath, A. Hamadeh, and D. Del Vecchio. Model reduction for a class of singularly perturbed stochastic differential equations. *American Control Conference,* 2015.

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INVITED SEMINARS (past year only)

- Symposium on Control of Network Systems, *Boston U*, 2014
- Network Science Workshop, *Berkeley*, 2014
- LabLinks (Cell Press), *MIT*, 2014