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QUANTITATIVE ANALYSIS, DESIGN, AND FABRICATION OF BIOSENSING AND BIOPROCESSING DEVICES IN LIVING CELLS

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Professor Domitilla Del Vecchio

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This project aims at designing sensing systems in bacteria E. coli by employing and re-engineering components from natural sensing 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.

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Professor Domitillla Del Vecchio
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Subject: Final Progress Statement to Dr. Hugh DeLong

Grant/Contract Title: QUANTITATIVE ANALYSIS, DESIGN, AND FABRICATION OF BIOSENSING AND BIOPROCESSING DEVICES IN LIVING CELLS
Grant/Contract Number: FA9550-10-1-0242

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 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.

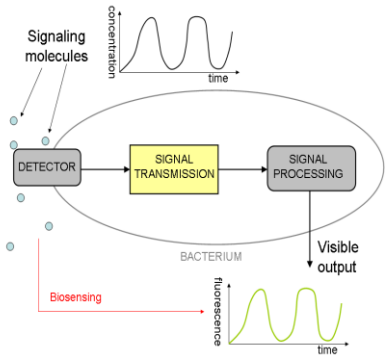

Figure 1: Natural Sensing System. In a cell, the signal transmission machinery is usually performed by networks of covalent modification cycles. The signal processing machinery (computation) is performed by genetic circuits.

Sensing through gene expression

We assembled the following circuit as a candidate sensing device composed of gene expression parts.

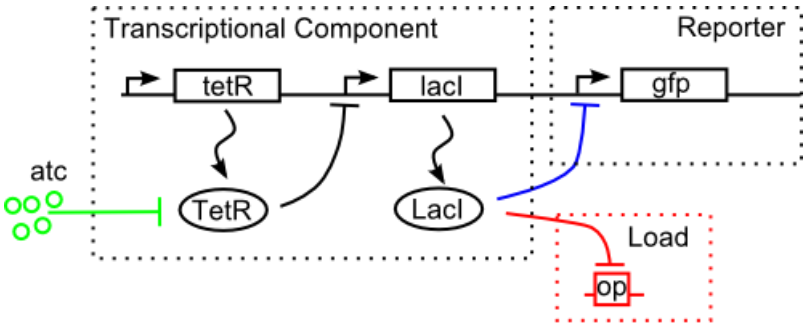

Fig 2. Sensing system composed of two transcriptional components and a reporter system (gfp). Signaling molecule atc is the input to be sensed and LacI protein responds to it by increasing when atc increases. The measured output is gfp, which decreases when atc increases. The "load" represents downstream processing systems that take LacI as an input. This system was assembled in *E. coli* cells in medium copy-number plasmids pACYC.

We characterized both the steady state transfer curve and the dynamic response of this system depending on whether the downstream processing (load) devices were present or not. This study is crucial to understand the effects of retroactivity from downstream processing systems on the biosensor performance. In fact, any biosensor will work when connected to downstream processing systems. Therefore, it is important to understand how its behavior characterized in isolation (without the downstream processing systems) is affected by the connection to its downstream clients.

Steady state transfer curve

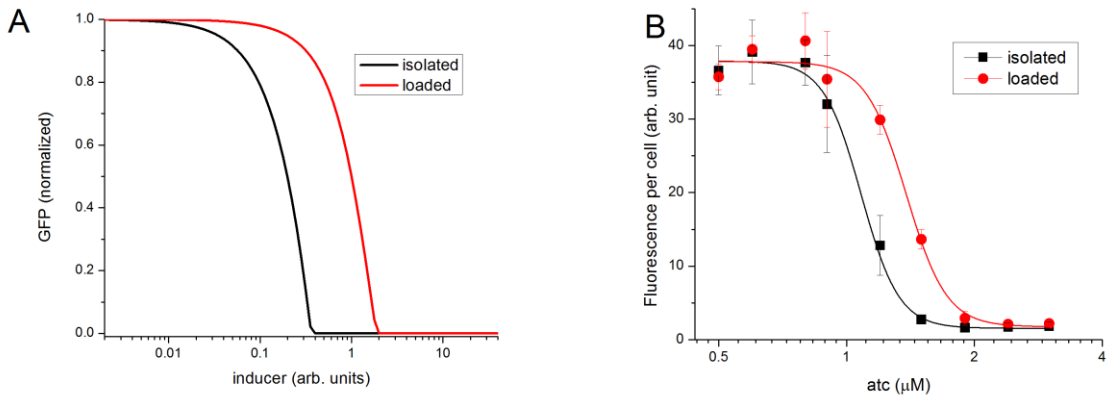

Fig 3. Loaded systems have a higher apparent dissociation coefficient with no change in the Hill coefficient. (A) Simulations show that the load increases the apparent dissociation coefficient of the response to the transcriptional component without changing the Hill coefficient. (B) Experimental data showing an increase of 30% on the apparent dissociation constant with no significant change in the Hill coefficient. Experimental data was fitted using non-linear regression on a repression-type Hill function model. The dissociation constant went from 1.08±0.02μM in the isolated system to 1.38±0.03μM in the loaded system. The Hill coefficient identified was 9.7±1.3 for the isolated system and 9.1±1.5 for the loaded system.

Figure 3 shows that the point of half maximal response of the sensor shifts to higher values. That is, in order to obtain the same response in the output, a higher input stimulation must be applied when the output of the sensor is used by other processes. This finding is crucial for designing biosensors that have a desired detection level. Only knowing the difference between the red and the black curve we can establish the sensing threshold for the device. This threshold will be much higher when we know that the sensor output is being used by other processes.

Dynamic Response to sudden input stimuli

Figure 4 shows that when the input stimulus is suddenly applied, the sensor presents a large delay in responding when the sensor output is connected to downstream clients. This is obviously undesirable because a sensor output is always going to be used by downstream clients.

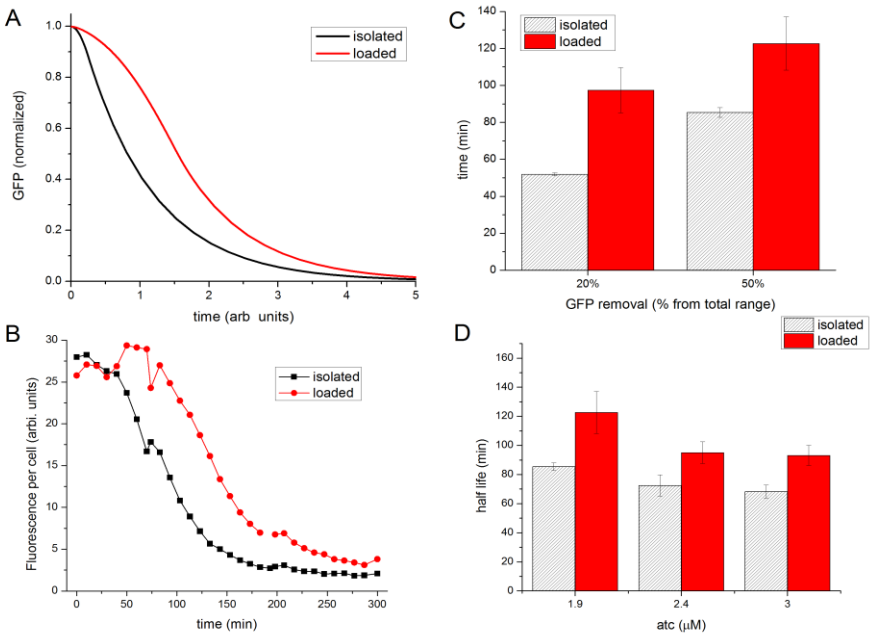

Fig 4. Load slows the response to induction by up to 40% in this circuit. (A) Simulation of the model in Figure 1 illustrates the effect of retroactivity on the dynamic response to addition of input. Note that load appears to cause a stronger lag earlier on the induction. The units in this simulation are nondimensionalized. Parameters used in this simulation are given in the supplementary information. (B) Experimental results show a good agreement with our model for transcriptional retroactivity. Upon the addition of 1.9μM atc, the average half-life of GFP (50% elimination) post-induction goes from 85±2 to 122±14min (43% change). (C) The slower response in the early stages of induction, can be quantified by comparing the delay at different elimination levels. The time it takes to remove 20% of the GFP presents a delay of 40min, slightly higher than the 37min delay due to load in the half-life value. (D) Higher levels of atc can decrease the half-life, but the delay caused by the load persists and as such pre-compensation strategies to avoid retroactivity may not work.

In contrast with the decrease of performance in steady state, this decreased performance in the dynamic response cannot be compensated in any way. It can be fixed only through the design of insulation devices, which use high gain feedback to perform reliably independently of downstream processes. This is what we are currently investigating in the new MIT grant.

Dynamic Response to sudden removal of input stimuli

While connection to downstream clients causes a degrade in the performance of the sensor response to sudden appearance of input stimuli, it causes an improvement in performance for the response to sudden removal of input stimulus. In fact, the response of the connected system (loaded) becomes faster. That is, the sensor detects earlier a decrease of the input stimulus when its output is used by downstream clients. This is shown in Figure 5.

Since a connected sensor performs worse in responding to sudden appearance of input stimuli and better in responding to sudden removal of input stimuli, the fundamental question arises of

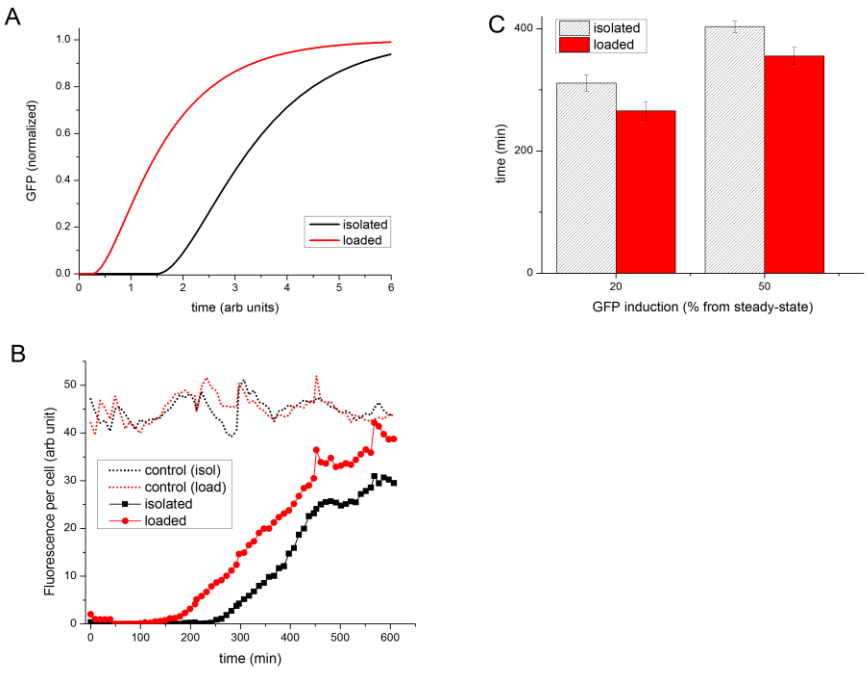

Fig 5. Load generates a faster response to removal of the input stimulation. (A) Simulation of the model from Figure 1 illustrates the effect of retroactivity on the dynamic response to removal of input stimulus. Note that the model predicts a decrease in the lag early. (B) Experimental results validate the results predicted by the model by showing that there is a consistent lag of 50min in the response to removal of inducer from cultures pre-induced with 3μM atc for 400min. The time to reach 50% of the steady-state level post-wash went down from 403±9min in the isolated system to 355±15min in the loaded system. (C) This column plot shows that the increase in the speed response occurs mainly in the early stages. The gap between isolated and loaded systems in the time to reach 20% and 50%of the steady-state are similar.

how we can optimize the sensor performance both in response to application and removal of input stimuli and independently of whether the sensor output is being used by downstream clients. This fundamental question is being addressed in the new MIT grant. An overview of this problem, the proposed solution, and the current status is included in the next section here.

Sensing through covalent modification

In the new MIT grant, we are designing and fabricating a semi-synthetic transmission system based on a phosphorylation cycle in *E. coli.* Specifically, Figure 6 shows the semi-synthetic transmission system based on phosphorylation. Specifically, the input signal is given by small signaling molecule aTc and the output is the phosphorylated NRII protein that is a transcription factor activating the glnA promoter. This output is measured indirectly by the fluorescent reporter superfolder GFP-lite, which fluoresces in green. The *E. coli* strain in which this is implemented is the 3.300LG strain, which is a double mutant of NRI and NRII in the 3.300 strain, so to prevent interference with the circuit behavior. Ideally, this system should provide a fast and sensitive GFP response to any change of the input stimulus atc, independently of

whether the output of this device is being used by downstream clients. In our earlier theoretical work, we have shown that this feature is guaranteed if the cycle has sufficiently high amplification and feedback gains. This can, in turn, be guaranteed if the amounts of substrate NRI and phosphatase NRII are sufficiently large. Hence (see Figure 6), we have placed the NRI and NRII phosphatase under the control of a constitutive promoter and a promoter inducible through IPTG, respectively. Increasing the amount of IPTG increases the amount of phosphatase, which increases the strength of the negative feedback. This allows us to tune the strength of the feedback gain, which is a key to attain a fast and reliable response to input stimuli, which is robust to the retroactivity from downstream clients that use NRI phosphorylated as an input.

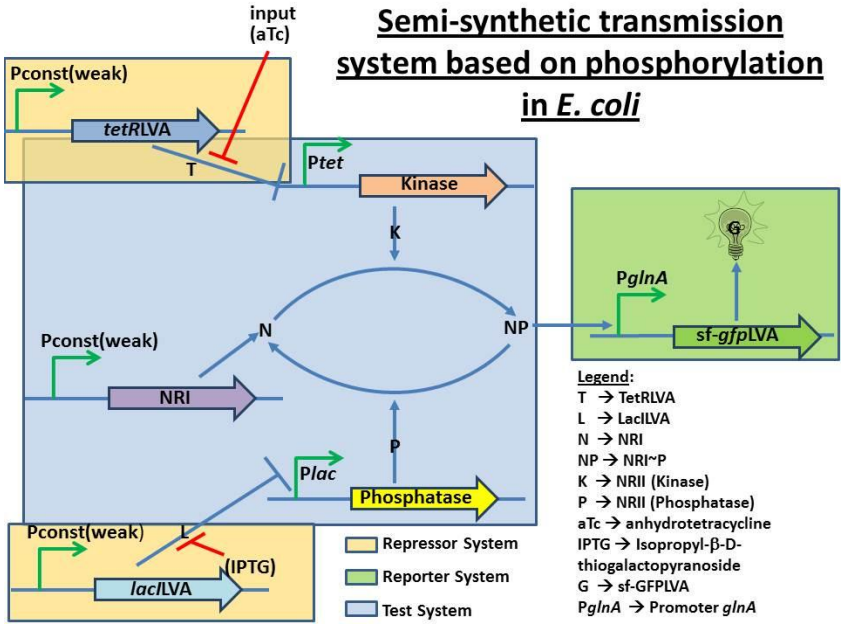

Fig 6. Implementation of a semi-synthetic transmission system based on phosphorylation in *E. coli.* The phosphorylation cycle is given by the NRI-NRIp (phosphorylated) cycle.

We successfully fabricated this system in pACYC plasmid and placed the load (glnA and glnK promoters) on pUC plasmid. We followed the bio-brick standard assembly procedure and followed a sequential cloning strategy. We specifically cloned one piece at the time in pACYC, sequenced the product, and performed functional testing to check the phenotype. All tests performed at each step of the construction process were successful. The final circuit genetic map is shown in Figure 7.

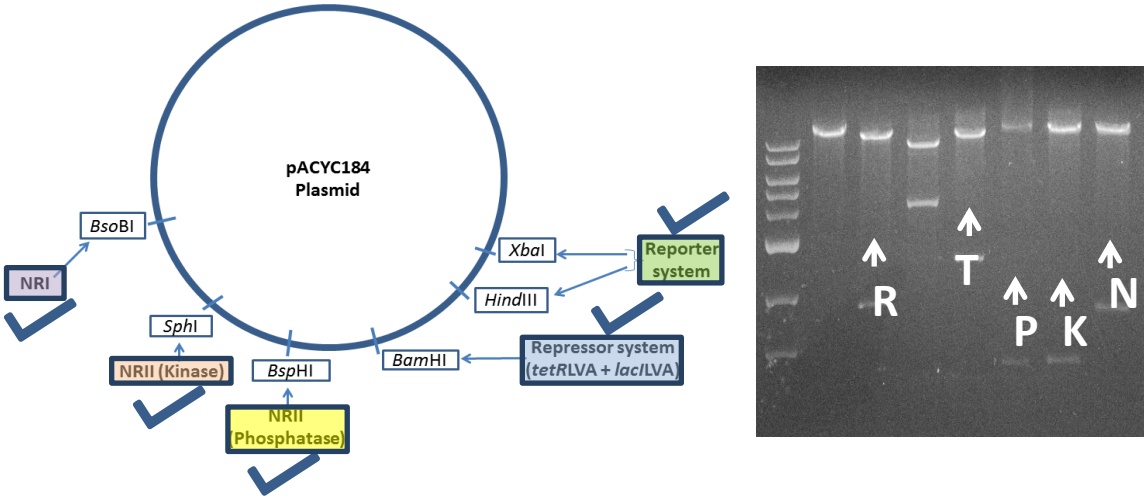

Fig 7. On the left, we show the restriction sites that were used to clone each of the components shown in Figure 6. On the right, we show a gel showing the length of the DNA segments obtained after restriction of the circuit on the left with each of the restriction enzymes indicated. The length of the various inserts matches the expected one. This, along with the several sequencing that we have done, shows that the circuit is correct.

PAPERS (past year only)

- P. Jiang, A. C. Ventura, S. D. Merajver, E. D. Sontag, A. J. Ninfa, and D. Del Vecchio, Load-induced Modulation of Signal Transduction Networks, *Science Signaling*, Vol. 4, Issue 194, p. ra67, 2011. (**This has an Editor's Choice in *Science* by L. Bryan Ray "Enlightening the load"**)

- H. R. Ossareh, A. C. Ventura, S. D. Merajver, and D. Del Vecchio, Long Signaling Cascades Tend to Attenuate Retroactivity *Biophysical Journal*, vol. 100(7):1617-1626, 2011.

- S. Jayanthi and D. Del Vecchio, *PLoS ONE*, Tuning Genetic Clocks through DNA Binding Sites vol 7(7), 2012.

- A. Gyorgy and D. Del Vecchio. Retroactivity to the Input and Thevenin's Theorem for Complex Gene Transcription Networks. *Proc. IEEE Conf. on Decision and Control*, 2012 (Invited).

ABSTRACTS (past year only)

- A. Gyorgy and D. Del Vecchio. Modularity in Complex Gene Transcrition Networks. *International Conference on Systems Biology*, Toronto, 2012

- K. Nilgiriwala, P. M. Rivera, and D. Del Vecchio. Insulation Mechanisms of *in vivo* Biomolecular Circuits. *International Conference on Systems Biology*, Toronto, 2012.

- S. Jayanthi and D. Del Vecchio. Impact of Retroactivity on Transcriptional Components. *The 5th Annual q-bio Conference on Cellular Information Processing*, Santa Fe, 2012.

- D. Del Vecchio. Engineering Insulation from Retroactivity of the Frequency Response of Covalent Modification Cycles. *IEEE Biomedical Circuits & Systems Conference*, San Diego, 2011. (Invited)

- D. Del Vecchio. A Control Theory Approach to Engineering Biomolecular Circuits. *33rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society* , Boston, 2011.

- P. Jiang, A. C. Ventura, S. Merajver, E. D. Sontag, A. J. Ninfa, and D. Del Vecchio. The Impact of Retroactivity on the Signal Processing of Transduction Networks. *The 5th Annual q-bio Conference on Cellular Information Processing*, Santa Fe, 2011.

- S. Jayanthi and D. Del Vecchio. Retroactivity changes the input/output steady-state characteristic of a transcriptional component. *SB5.0: The 5th International Meeting on Synthetic Biology*, Stanford, 2011

INVITED SEMINARS (past year only)

- Bioengineering Seminar, Tufts University, September 2011
- Mechanical Engineering Colloquium, UIUC, September 2011
- Workshop on Robustness in Biological Systems, IEEE Multiconference on Systems and Control, October 2011
- Synthetic Biology Center Kick-off Workshop, MIT, December 2011
- Mechanical Engineering Colloquium, Princeton University, April 2012
- Workshop on design, optimization and control in systems and synthetic biology, INRIA, France, June 2012
- Workshop on Verification in Systems Biology, CAV 2012, Berkeley

NEWS: Living cells say: Can you hear me now? by D. L. Chandler *MIT News Office*, November 2011

PLENARIES: A control Theory Approach to Engineering Biomolecular Circuits. *American Control Conference*, 2011 (Semi-plenary speaker in honor of the Donald P. Eckman Award by the AACC)