

Analog Transistor Models of Bacterial Genetic Circuits

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Abstract—We show that compact analog current-mode circuits are effective at quantitatively modeling the behavior of genetic circuits. We present experimental biological data from genetic activator (PBAD) and repressor (PlacO) promoter circuits in *E. coli*. Simple subthreshold cascaded-differential-pair transistor circuits have input-output characteristics that quantitatively represent this data. Such foundational analog circuits can provide efficient conceptual, modeling, and simulation tools for the design and analysis of circuits in synthetic and systems biology.

I. INTRODUCTION

Due to the deep mathematical similarities between stochastic subthreshold electronic current flow and stochastic molecular flux in chemical reactions illustrated in Fig. 1, digitally programmable analog circuits can efficiently represent molecular circuits within the cell. Such circuits have been demonstrated on working VLSI chips that represent programmable mass-action behavior and genetic logic behavior [1, 2, 3]. Several constraints such as flux balance analysis and thermodynamic energy balance in biochemical circuits are automatically satisfied as Kirchhoff's Current Law and Kirchhoff's Voltage Law in the corresponding electronic representations respectively. Hence, *highly computationally intensive* molecular circuits and networks with DNA-protein interactions and protein-protein interactions can be modeled, designed, and *rapidly simulated* in analog electronic circuits on chips that also naturally model their stochastics, dynamics, and loading interactions [1]. Such an approach avoids overly simplified logic-gate analogies with too much abstraction or overly complex differential-equation approaches with too little abstraction. Analog electronic circuit design techniques and tools can provide a valuable conceptual and simulation framework for scaleably complex systems biology and synthetic biology in the future just as they have in the semiconductor industry in the past. In this work, we provide examples of simple subthreshold electronic circuits that model experimental data from activator and repressor genetic circuits in *E. coli*, the most well studied bacterium to date [4].

For readers unfamiliar with bacterial genetic circuits, we provide a simplified review based on Fig. 2: inducer molecules such as S_x induce state changes in activator

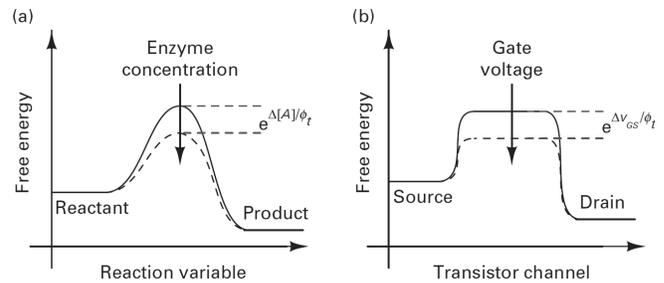


Figure 1. Analogies between (a) molecular flux in chemical reactions and (b) electronic current flow in subthreshold transistors. The mean current flow and stochastics of Poisson flow are similar in both domains [1].

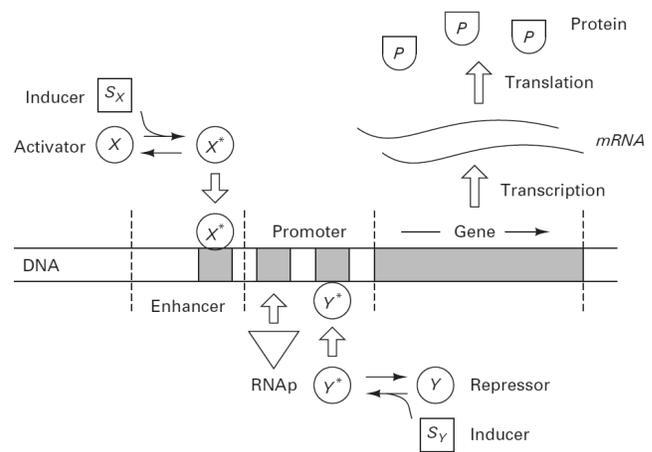


Figure 2. A simplified overview of the processes of induction, transcription, and translation in a bacterial genetic circuit.

molecules from X to X^* by binding to them. The activator molecules then bind to DNA at enhancer sites and increase the rate of transcription of DNA to mRNA by the enzyme RNA polymerase (RNAP). Inducer molecules such as S_y induce state changes in repressor molecules from Y to Y^* by binding to them. These repressor molecules then bind to DNA at operator sites and decrease the rate of transcription. The translation of mRNA to protein is performed by ribosomal binding to mRNA and is not shown in Fig. 2.

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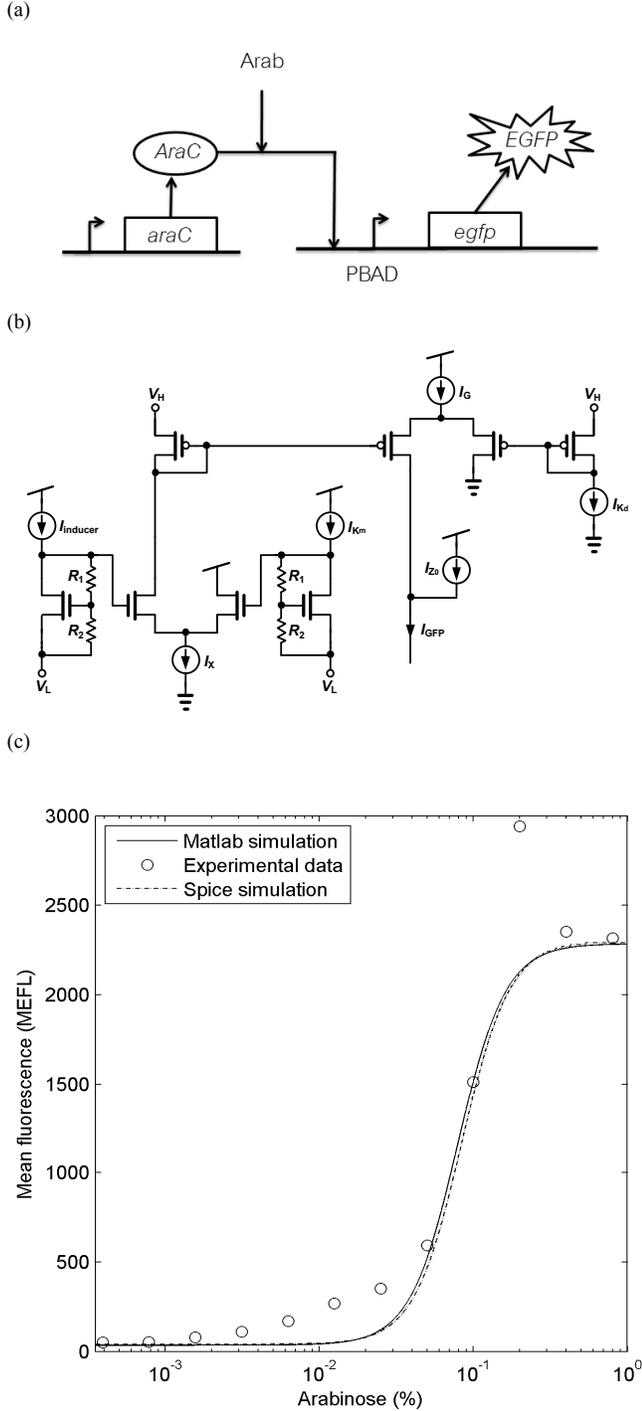


Figure 3. (a) Representation of the PBAD genetic circuit in *E. coli*. (b) The subthreshold electronic circuit used to represent its operation. (c) Fits to biological fluorescence data by MATLAB functions and SPICE simulations of the circuit of (b).

Simplified analog transistor models are useful in the design and analysis of practical electronic systems since they quantitatively represent their behavior. Similarly, we shall show that they are useful in quantitatively representing the functional characteristics seen in cells. In fact, even relatively

crude digital-logic approximations of genetic circuits have proven useful in the past in understanding simple cellular systems [5]. However, such approximations are increasingly proving to be inadequate in understanding more complex biological systems that experimentally exhibit analog and noisy behavior rather than purely digital behavior. Fortunately, the deep similarity behind subthreshold electronic current flow and molecular reaction dynamics shown in Fig. 1 enables an efficient mapping between genetic circuits and analog subthreshold or bipolar electronic circuits [1]. In this work, we reveal for the first time compact analog subthreshold transistor circuits that efficiently represent the experimental behavior of bacterial genetic circuits.

This paper is organized as follows. In Section II, we discuss an experimental activator circuit in the bacterium *E. coli* based on an *AraC* activator protein and a transistor circuit analog used to represent it. In Section III, we discuss an experimental repressor circuit in *E. coli* based on a *LacI* repressor protein and a transistor circuit analog used to represent it. In Section IV, we provide details of the experimental methods used in this work. In Section V, we conclude with a brief discussion of the potential benefits of this work.

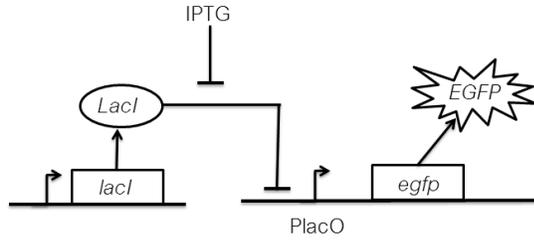
II. A GENETIC ACTIVATOR CIRCUIT

Fig. 3a shows a schematic representation of a genetic circuit constructed on a plasmid that was transfected into *E. coli* using standard genetic techniques [4]. The *AraC* activator protein is constitutively expressed; its activation and consequent ability to stimulate transcription from the PBAD promoter is controlled by an Arabinose (Arab) inducer. The reporter protein EGFP enables monitoring of the expression level from the PBAD promoter via standard fluorescence techniques. The subthreshold transistor circuit shown in Fig. 3b models the behavior of the genetic circuit of Fig. 3a. The cascaded differential pairs quantitatively model the Michaelis-Menten binding equations of the inducer to the activator molecule and the activator molecule to the DNA, respectively. The R_1 - R_2 resistive divider enables the Hill coefficient of the inducer-activator binding to be different from that of the activator-DNA binding. The current I_{Z_0} represents the basal rate of transcription when the inducer is not present such that I_{GFP} , the total observed fluorescence proportional to protein level of EGFP in Fig. 3a, is given by

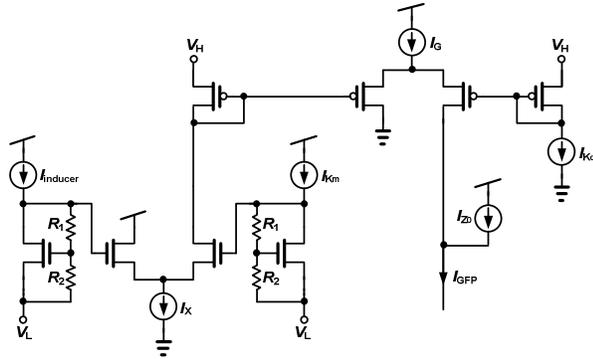
$$I_{GFP} = \frac{I_G}{1 + \frac{I_{K_d}}{I_X} \left(1 + \left(\frac{1}{I_{inducer} / I_{K_m}} \right)^m \right)} + I_{Z_0}, \quad (1)$$

where $m = (R_1 + R_2)/R_2$, I_X represents the total activator concentration, I_{K_m} is the Michaelis-Menten constant for the inducer-activator binding, I_{K_d} is the Michaelis-Menten constant for the activator-DNA binding, $I_{inducer}$ is the inducer concentration, and I_G represents a combined rate of transcription-factor-dependent protein production based on combined RNA polymerase and ribosomal activity. Remarkably, if, in the biological measurements, we set $x = X/K_d$, $I = [Arab]/K_m$, Z_0 is the basal level of fluorescence, and G is a constant that represents combined RNA polymerase

(a)



(b)



(c)

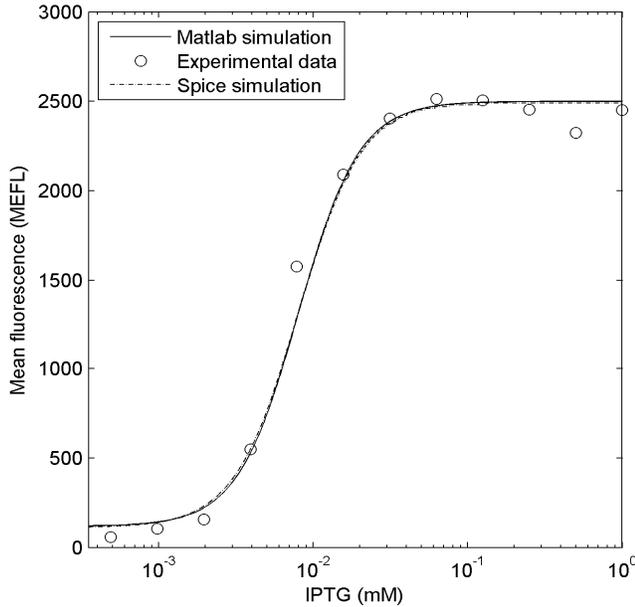


Figure 4. (a) Representation of the PLacO genetic circuit in *E. coli*. (b) The subthreshold electronic circuit used to represent its operation. (c) Fits to biological fluorescence data by MATLAB functions and SPICE simulations of the circuit of (b).

and ribosomal protein production rate dependent on the transcription factor, we find that the observed fluorescence Z is described by an equation that is identical in form to (1) and derived from prior modeling work described in [6]:

$$Z = \frac{G}{1 + \frac{1}{x} \left(1 + \left(\frac{1}{I} \right)^m \right)} + Z_0. \quad (2)$$

Fig. 3c illustrates that the MATLAB fit to (2) and the SPICE simulation of Fig. 3b, which is well described by (1), both generate identical fits to observed experimental data for the variation of fluorescence with changing Arabinose inducer level. The SPICE fit is plotted after proportional conversion of current to chemical concentration with 400 nA of $I_{inducer}$ corresponding to 1 % concentration of the Arabinose inducer, and 1 nA of I_{GFP} corresponding to 100 observed fluorescence units. The circuit parameters of Fig. 3b corresponding to the fits in Fig. 3c were $(R_1+R_2)/R_2 = m = 2.8$, $I_{K_m} = 60$ nA, $I_X = 50$ nA, $I_{K_d} = 10$ nA, $I_G = 27$ nA, and $I_{Z_0} = 0.35$ nA, $V_L = 1$ V, and $V_H = 4$ V with the power supply voltage being 5 V. The value of $I_{inducer}$ was swept from 0.04 nA to 400 nA and all currents were maintained under 500 nA to ensure that all transistors operated in the subthreshold regime ($V_{T0} = 0.71$ V for NMOS transistors and $V_{T0} = -0.92$ V for PMOS transistors).

III. A GENETIC REPRESSOR CIRCUIT

Fig. 4a shows a schematic representation of a genetic circuit constructed on a plasmid that was transfected into *E. coli* using standard genetic techniques [4]. The LacI repressor protein is constitutively expressed; its inactivation and consequent ability to stimulate transcription from the PLacO promoter is controlled by the IPTG inducer. The reporter protein EGFP enables monitoring of the expression level from the PLacO promoter via standard fluorescence techniques. The subthreshold transistor circuit shown in Fig. 4b models the behavior of the genetic circuit of Fig. 4a. The cascaded differential pairs quantitatively model the Michaelis-Menten binding equations of the inducer to the repressor molecule and the repressor molecule to the DNA, respectively. The R_1 - R_2 resistive divider enables the Hill coefficient of the inducer-repressor binding to be different from that of the repressor-DNA binding. The current I_{Z_0} represents the basal rate of transcription when the inducer is not present such that I_{GFP} , the total observed fluorescence proportional to protein level of EGFP in Fig. 4a, is given by

$$I_{GFP} = \frac{I_G}{1 + \frac{I_X / I_{K_d}}{1 + \left(I_{inducer} / I_{K_m} \right)^m}} + I_{Z_0}, \quad (3)$$

where $m = (R_1+R_2)/R_2$, I_X represents the total repressor concentration, I_{K_m} is the Michaelis-Menten constant for the inducer-repressor binding, I_{K_d} is the Michaelis-Menten constant for the repressor-DNA binding, $I_{inducer}$ is the inducer concentration, and I_G represents a combined rate of transcription-factor-dependent protein production based on combined RNA polymerase and ribosomal activity. Remarkably, if, in the biological measurements, we set $x = X/K_d$, $I = [IPTG]/K_m$, Z_0 is the basal level of fluorescence, and G is a constant that represents combined RNA polymerase and ribosomal protein production rate dependent on the transcription factor, we find that the observed fluorescence Z

is described by an equation that is identical in form to (3) and derived from prior modeling work described in [6]:

$$Z = \frac{G}{1 + \frac{x}{1 + I^m}} + Z_0. \quad (4)$$

Fig. 4c illustrates that the MATLAB fit to (4) and the SPICE simulation of Fig. 4b, which is well described by (3), both generate identical fits to observed experimental data for the variation of fluorescence with changing IPTG inducer level. The SPICE fit is plotted after proportional conversion of current to chemical concentration with 500 nA of $I_{inducer}$ corresponding to 1 mM concentration of IPTG, and 1 nA of I_{GFP} corresponding to 100 observed fluorescence units. The circuit parameters of Fig. 4b corresponding to the fits in Fig. 4c were $(R_1 + R_2)/R_2 = m = 2.2$, $I_{Km} = 1$ nA, $I_X = 100$ nA, $I_{Kd} = 5$ nA, $I_G = 25$ nA, and $I_{Z0} = 0$ nA. The value of $I_{inducer}$ was swept from 0.05 nA to 500 nA.

IV. EXPERIMENTAL METHODS

A. Strains and growth conditions

All manipulations were performed in the *E. coli* strain MG1655, kept in a 20% glycerol suspension at -80°C . The cells were grown overnight with shaking at 37°C in Luria Bertani (LB) broth containing 50 mg/liter of ampicillin. DNA-modifying enzymes were purchased from New England Biolabs. Oligonucleotides were synthesized by Integrated DNA Technology and used unpurified. PCR purification and Plasmid preparation were performed using QIAGEN kits. Plasmids were sequenced by GENEWIZ Technical. L-arabinose (Arab) and Isopropyl β -D-1-thiogalactopyranoside (IPTG) were supplied by Sigma-Aldrich.

The cells hosting the appropriate plasmids were incubated in 3 ml of LB broth at 37°C overnight in culture tubes containing 50 mg/liter of ampicillin, without inducers present. The cultures were then diluted 100-fold in fresh LB broth in 96-well plate format with ampicillin and the appropriate inducer. They were shaken at 37°C until the early exponential growth phase (optical density of 0.12 at 600 nm).

B. Data Collection

Flow cytometry was used to collect data. All data were obtained using the BD FACS LSR2, and contained at least 10,000 events. Events were gated by forward and side scattering using Cyflogic (<http://www.cyflogic.com/>) software. The geometric means of the fluorescence distributions were calculated. The auto-fluorescence value of *E. coli* cells with no plasmid was subtracted from these values to give the fluorescence values reported in this study.

C. Circuit Simulation

The SPICE circuit simulations were performed with Cadence design tools. Transistor parameters corresponded to

those in an ON-Semiconductor $0.5\mu\text{m}$ process available through MOSIS, a chip prototyping vendor. All transistors were sized at $60\mu\text{m}/3\mu\text{m}$ and operated in the subthreshold regime.

V. DISCUSSION

The physically intuitive symmetry of the electronic circuits of Fig. 3b and Fig. 4b, which model the genetic activator circuit of Fig. 3a and the genetic repressor circuit of Fig. 4a, respectively, should be noted: In the activator circuit of Fig. 3b, the differential-pair arm currents that lead to increased transcription correspond to increased activation of the transcription factor and increased activator-DNA binding just as in the genetic circuit of Fig. 3a. In the repressor circuit of Fig. 4b, the differential-pair arm currents that lead to increased transcription correspond to decreased activation of the transcription factor and decreased repressor-DNA binding just as in the genetic circuit of Fig. 4a. Both circuits lead to increasing transcription with increasing inducer level in electronics and in biology.

We have shown that the simple *cytomorphic* or cell-inspired electronic circuits described here can be directly and easily validated in biological experiments. Thus, they can have immediate impact in the design, analysis, and rapid simulation of molecular circuits in cell biology [1]. In the future, they may also lead to novel hybrid analog-digital electronic architectures that are inspired by circuits in cells [1].

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