Ratiometric logic in living cells via competitive binding of synthetic transcription factors

Samuel D. Perli
Gladstone Institutes, 1650 Owens St, San Francisco, CA
samuel.perli@gladstone.ucsf.edu

Timothy K. Lu
Synthetic Biology Group (SBG), MIT Research Laboratory of Electronics (RLE), Cambridge, MA
timlu@mit.edu

ACM Reference format:
Samuel D. Perli and Timothy K. Lu. 2017. Ratiometric logic in living cells via competitive binding of synthetic transcription factors. In *Proceedings of NANOCOM '17, Washington D.C., DC, USA, September 27–29, 2017,* 4 pages.
https://doi.org/10.1145/3109453.3124360

1 ABSTRACT

Although there have been a flurry of designs in the recent past describing implementations of digital logic in living cells, computational elements that perform analog operations such as division and subtraction are scarce. By employing the principle of competitive binding between different DNA binding proteins, we present a novel approach towards ratiometric computation in living cells. After developing a quantitative model to analyze our design, we build and experimentally characterize our system in *Saccharomyces cerevisiae*. Our work demonstrates the feasibility of performing analog computation in eukaryotic cells and will potentially enable the design of more sophistated gene networks.

2 INTRODUCTION

Over the past couple of decades, several synthetic biological parts have been developed towards enabling computation in living cells. These include synthetic transcription factors [8], DNA recombinases [2], RNA switches [17], ribozymes [12], and chimeric proteins [4]. A wide range of synthetic computational devices have been built in living cells employing these parts. To name a few, we currently have oscillators [6], logic gates [15] and counters [9]. However, multiple such digital devices are needed to be integrated in cells in order to perform relatively complex computation such as division or subtraction. Assembling many molecular devices in single cells can be challenging due to the limited resources present in each cell.

An alternative approach to computation in living cells is the analog approach wherein the underlying mathematical principles of biochemical processes can be harnessed to implement complex computation. For example, by building a negative auto-regulatory feedback loop, the authors in [11] achieved a linear input-output transfer function. Using the principles of positive and negative feedback, Daniel et. al., from our lab [3] built wide log-linear transfer functions and implemented power law relationships between the input and output. They also implemented the divison operation between extracellular small molecules. Recently, intracellular analog memory devices that can record the magnitude and/or duration of biological activities have been built both in bacteria and human cells [7, 13].

Intracellular ratios of proteins are often key indicators of celluar state. For example, the ratio of intracellular protein levels of Bcl-2 and Bax is a prognosticator of clinal resistance and apoptosis potential in cancer cells [14, 16] while the ratio of the CRY1 and CRY2 protein levels determines the circadian clock period length [10]. However, there are currently no known synthetic gene circuits that can measure and report such ratios.

3 A SYNTHETIC TRANSCRIPTION FACTOR RATIOMETER

We have designed a synthetic gene circuit that expresses a reporter gene (Blue Fluorescent Protein - BFP) at levels that are expected to be a function of the ratio of two input proteins. This circuit employs the principle of competitive binding wherein an activating transcription factor and a competing transcription factor, both bind the same target DNA in the promoter of the BFP reporter (Fig. 1A). The binding of the activating transcription factor activates the expression of BFP while the binding of the competing transcription factor occludes the activating transcription factor from binding to the target DNA in the promoter of BFP. We surmised that the activity of the promoter directly depends upon the intracellular levels of the two transcription factors and hence the BFP expression level must correspond to the ratio of the input transcription factors.

3.1 A quantitative model for competitive binding of synthetic transcription factors

The dynamics of the transcription factor ratiometer can be described by the following affinity equations:

$$D + TF \underset{k_{d-}}{\overset{k_{d+}}{\rightleftharpoons}} D\text{-}TF \xrightarrow{k_p} P$$

$$D + I \underset{k_{i-}}{\overset{k_{i+}}{\rightleftharpoons}} D\text{-}I$$

where TF is the activating transcription factor, I is the competing transcription factor, D is the DNA binding site upstream of the reporter gene and P is the protein expressed. $k_{d+}, k_{d-}, k_{i+}, k_{i-}$ and k_p are the associated rate constants as shown above. According to

NANOCOM '17, September 27–29, 2017, Washington D.C., DC, USA

ACM ISBN 978-1-4503-4931-4/17/09…$15.00
https://doi.org/10.1145/3109453.3124360

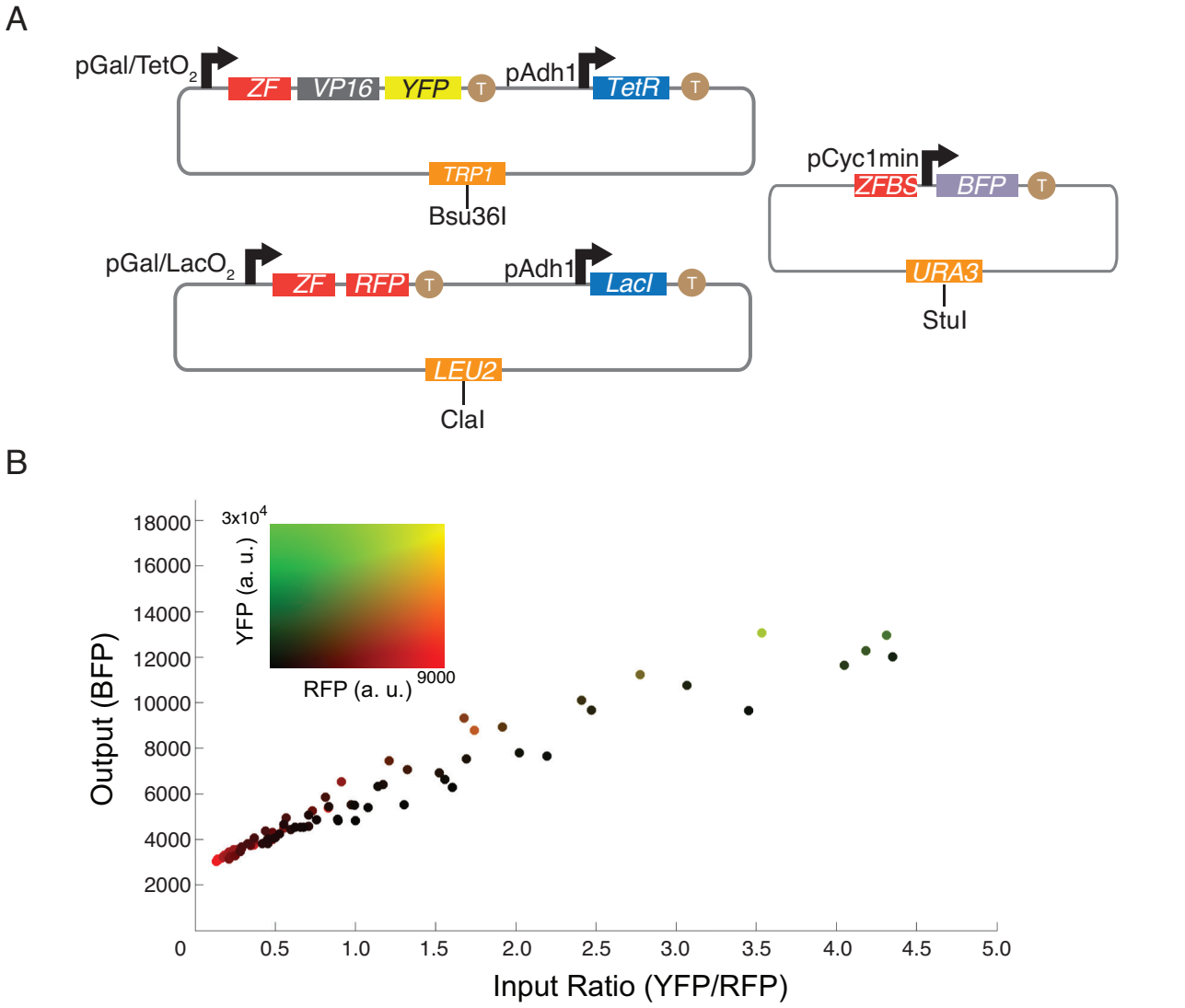

Figure 1: ZF ratiometer characterization in *S. cerevisiae* A) The levels of the output protein BFP is designed to be a function of the ratio of the levels of synthetic transcription factors ZF-VP16-YFP and ZF-RFP. B) The mean BFP levels measured are plotted against the ratio of the mean levels of YFP and RFP. Each data point plotted is color coded to capture the relative levels of YFP and RFP as indicated in the inset.

the law of mass action, we have

$$\frac{d}{dt}[D\text{-}TF] = k_{d+}[D][TF] - k_{d-}[D\text{-}TF] - k_p[D\text{-}TF]$$
$$\frac{d}{dt}[D\text{-}I] = k_{i+}[D][I] - k_{i-}[D\text{-}I]$$

In addition, since the total amount of reporter genes per cell, D_{Tot} is constant in our case, we have

$$[D_{Tot}] = [D\text{-}TF] + [D] + [D\text{-}I] \quad (1)$$

At the steady state, we set $\frac{d}{dt}[D\text{-}TF] = 0$ and $\frac{d}{dt}[D\text{-}I] = 0$. This results in

$$[D\text{-}TF] = \frac{k_{d+}}{(k_{d-} + k_p)}[D][TF] = \frac{[D][TF]}{K_d} \quad \left(K_d = \frac{k_{d-} + k_p}{k_{d+}}\right)$$
$$[D\text{-}I] = \frac{k_{i+}}{ki-}[D][I] = \frac{[D][I]}{K_i} \quad \left(K_i = \frac{k_{i-}}{k_{i+}}\right)$$

From equation (1), we have

$$[D_{Tot}] = [D\text{-}TF]\left(1 + \frac{K_d}{[TF]} + \frac{K_d}{K_i}\frac{[I]}{[TF]}\right)$$
$$\implies [D\text{-}TF] = \frac{[D_{Tot}][TF]}{\left([TF] + K_d + \frac{K_d}{K_i}[I]\right)}$$

The rate of expression of the reporter gene, v is given by $k_p[D\text{-}TF]$

$$v = \frac{k_p[D_{Tot}][TF]}{\left([TF] + K_d + \frac{K_d}{K_i}[I]\right)} = \frac{v_{max}\rho}{\left(\rho + \frac{K_d}{[I]} + \frac{K_d}{K_i}\right)}$$

where $v_{max} = k_p \times [D_{Tot}]$ is the maximum possible rate of expression of the reporter gene and $\rho = \frac{[TF]}{[I]}$ is the ratio between the intracellular concentrations of the activating transcription factor and the competing transcription factor.

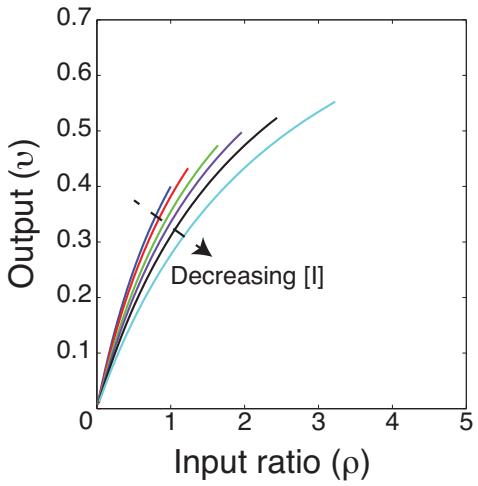

Figure 2: Transcription factor ratiometer model. The rate of production of the output protein (v) is plotted as a function of the input transcription factor ratio (ρ) for different concentrations of the competing transcription factor (I).

In Fig. 2, we plot the input-output relationship for the transcription factor ratiometer as predicted by the above model. Assuming $K_d \simeq K_i$ (the activating transcription factor and the competing transcription factor are comprised of the same zinc finger), we observe that $v \propto \rho$ when $K_d >> [I]$. In other words, the rate of production of the output protein is directly proportional to the input transcription factor ratio when $K_d >> [I]$ i.e, for transcription factors that have relatively low affinity to their target DNA.

3.2 Experimental validation of transcription factor ratiometer

In our specific implementation in *S. cerevisiae*, the input synthetic transcription factors were built by using Zinc Finger (ZF) DNA binding proteins that target a specific 9 bp DNA sequence present in the promoter of BFP. The activating ZF transcription factor (ZF-TF) is built by fusing the ZF domain to VP16, a commonly used eukaryotic transcription activating domain [1]. The competing ZF-TF does not contain VP16. Both the activating and the competing ZF-TFs are also tagged by the fluorescent proteins YFP and RFP to enable measuring their intracellular concentrations. The expression of both ZF-TFs is designed to be independently inducible using the small molecules anhydrotetracycline (aTc) and isopropyl β-D-1-thiogalactopyranoside (IPTG). The gene cassettes encoding the transcription factors and the reporter were introduced in to the genome of *S. cerevisiae*. The intracellular levels of transcription factors were varied by growing cells in media containing different concentrations of aTc and IPTG.

In Fig. 1B, we plot the measured levels of the reporter (BFP) as a function of the ratio of YFP and RFP per cell. We notice a strong correlation between the output level and the ratio of input transcription factors. Moroever, the correlation persists over ratios greater or lesser than one. We also notice that the output is a strong function of the ratio even when the inputs vary over a wide dynamic range.

4 CONCLUSIONS

We have built and characterized an intracellular ratiometer that reports on the ratios of transcription factors. Our design is guided by an underlying mathematical characterization of transcription factor activity in conditions of competitive binding. Compared with digital designs, our design employs much fewer parts, emphasizing the power of analog circuit design. However, while implementing analog gene circuits towards general purpose computation in living cells, one has to be able to relate the underlying mathematical principles of biochemical process to the computation of interest.

5 MATERIAL AND METHODS

We used the *S. cerevisiae* strain YPH500 (*MATα ura*3-52 *lys*2-801_*amber ade*2-101_*ochre trp*1-Δ63 *his*3-Δ200 *leu*2-Δ1) in our above experiment. The ZF-TFs were cloned into the vector backbones pRS404 and pRS405 to be expressed via the pTPGI and pLOGI promoters [5]. The reporter plasmid was cloned into the pRS406 backbone. All of the plasmid DNA sequences are available on request. The plasmids were linearized with restriction sites StuI, Bsu36I and ClaI and integrated in to YPH500 via homologous recombination. Successful single copy integrant colonies were picked following prototrophic selection. Yeast transformations and fluorescence measurements were performed as described previously [8, 12].

REFERENCES

[1] R. R. Beerli, D. J. Segal, B. Dreier, and C. F. Barbas. Toward controlling gene expression at will: specific regulation of the erbB-2/HER-2 promoter by using polydactyl zinc finger proteins constructed from modular building blocks. *Proc. Natl. Acad. Sci. U.S.A.*, 95(25):14628–14633, Dec 1998.

[2] J. Bonnet, P. Subsoontorn, and D. Endy. Rewritable digital data storage in live cells via engineered control of recombination directionality. *Proc. Natl. Acad. Sci. U.S.A.*, 109(23):8884–8889, Jun 2012.
[3] R. Daniel, J. R. Rubens, R. Sarpeshkar, and T. K. Lu. Synthetic analog computation in living cells. *Nature*, 497(7451):619–623, May 2013.
[4] J. E. Dueber, B. J. Yeh, K. Chak, and W. A. Lim. Reprogramming control of an allosteric signaling switch through modular recombination. *Science*, 301(5641):1904–1908, Sep 2003.
[5] T. Ellis, X. Wang, and J. J. Collins. Diversity-based, model-guided construction of synthetic gene networks with predicted functions. *Nat. Biotechnol.*, 27(5):465–471, May 2009.
[6] M. B. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335–338, Jan 2000.
[7] F. Farzadfard and T. K. Lu. Genomically Encoded Analog Memory with Precise In vivo DNA Writing in Living Cell Populations. *Science*, 346, November 2014.
[8] F. Farzadfard, S. D. Perli, and T. K. Lu. Tunable and multifunctional eukaryotic transcription factors based on CRISPR/Cas. *ACS Synth Biol*, 2(10):604–613, Oct 2013.
[9] A. E. Friedland, T. K. Lu, X. Wang, D. Shi, G. Church, and J. J. Collins. Synthetic gene networks that count. *Science*, 324(5931):1199–1202, May 2009.
[10] Y. Li, W. Xiong, and E. E. Zhang. The ratio of intracellular CRY proteins determines the clock period length. *Biochem. Biophys. Res. Commun.*, 472(3):531–538, Apr 2016.
[11] D. Nevozhay, R. M. Adams, K. F. Murphy, K. Josic, and G. Balazsi. Negative autoregulation linearizes the dose-response and suppresses the heterogeneity of gene expression. *Proc. Natl. Acad. Sci. U.S.A.*, 106(13):5123–5128, Mar 2009.
[12] L. Nissim, S. D. Perli, A. Fridkin, P. Perez-Pinera, and T. K. Lu. Multiplexed and programmable regulation of gene networks with an integrated RNA and CRISPR/Cas toolkit in human cells. *Mol. Cell*, 54(4):698–710, May 2014.
[13] S. D. Perli, C. H. Cui, and T. K. Lu. Continuous genetic recording with self-targeting CRISPR-Cas in human cells. *Science*, 2016.
[14] M. Raisova, A. M. Hossini, J. Eberle, C. Riebeling, T. Wieder, I. Sturm, P. T. Daniel, C. E. Orfanos, and C. C. Geilen. The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. *J. Invest. Dermatol.*, 117(2):333–340, Aug 2001.
[15] P. Siuti, J. Yazbek, and T. K. Lu. Synthetic circuits integrating logic and memory in living cells. *Nat. Biotechnol.*, 31(5):448–452, May 2013.
[16] K. E. Williamson, J. D. Kelly, P. W. Hamilton, D. McManus, and S. R. Johnston. Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br. J. Cancer*, 78(4):553–554, Aug 1998.
[17] M. N. Win and C. D. Smolke. Higher-order cellular information processing with synthetic RNA devices. *Science*, 322(5900):456–460, Oct 2008.