Analog synthetic gene networks

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ABSTRACT

In analog synthetic biology, the underlying mathematical principles of various biological processes is harnessed to perform complex computation. Here, we review key synthetic gene circuits that implement analog computation in living cells and propose biological constructs that can implement further novel analog computation.

CCS Concepts

•**Hardware → Biology-related information processing;**

Keywords

Synthetic biology; Analog synthetic gene circuits

1. INTRODUCTION

The field of synthetic biology is currently sixteen years old and has witnessed the development of several synthetic gene circuits with applications ranging from cellular computing to therapeutics [4]. Drawing from the discipline of Electrical Engineering, two classic paradigms for synthetic gene circuit logic and design have emerged: Digital and Analog. In the digital paradigm, input and output signals such as concentrations, redox potentials or electic voltages are arbitrarily thresholded and classified into the 'ON' and 'OFF' states. Complex computation is achieved by interconnecting several small switches that can transition between the 'ON' and 'OFF' states.

In the analog paradigm, instead of using thresholded values, the whole range of input and output signals is used for computation. Computation in the analog paradigm is performed by implementing mathematical functions using the underlying biochemical and biophysical principles of interacting molecules.

2.1 Repressilator

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One of the first synthetic gene circuit to be built was an analog circuit named the repressilator [3]. Three bacterial genes coding for repressor proteins (*lacI, tetR* and *cI*) were arranged in a configuration such that each of the proteins would repress the expression of either of the other two genes. The output expression of each of the genes then would exhibit an oscillatory pattern as governed by the following six coupled first-order differential equations [3]:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_i^n)} + \alpha_0; \frac{dp_i}{dt} = -\beta(p_i - m_i)$$

where $i = lacI, tetR, cI$ and $j = cI, lacI, tetR$. p_i represents the protein concentrations and m_i represents the mRNA concentrations. α_0 represents the leakiness of the promoter, β denotes the ratio of the protein decay rate to the mRNA decay rate; and n is the Hill coefficient. It turns out that in general, cyclic transcriptional feedback loops exhibit oscillations if they contain a single repressor and a single activator or an odd number of at least three repressors.

2.2 Band-detect networks

The authors in [1] used three bacterial repressors $lacI$, $lacI_{M1}$ and cI arranged in a feed-forward loop to achieve band-detect activity. In the designed circuit, the expression of *gfp* is activated only at moderate but not high or low concentrations of AHL. Successful performance of the circuit is achieved by tuning the binding affinity of $lacI_{M1}$ to its operator. By fusing different fluorescent proteins as outputs, the authors engineer bacteria containing the band-detect circuit to form a bullseye pattern. The following equations [1] describing a five-species model captures the band-detect activity, wherein $gfp, lacI, cI, luxR/AHL$ complex and AHL are represented by G, L, C, R and A respectively:

$$\frac{dG}{dt} = \frac{\alpha_G}{1+(L/\beta_L)^{\eta^1}} - \gamma_G G$$

$$\frac{dL}{dt} = \frac{\alpha_{L1}}{1+(C/\beta_C)^{\eta^2}} + \frac{\alpha_{L2}.R^{\eta^3}}{(\theta_R)^{\eta^3}+R^{\eta^3}} - \gamma_L L$$

$$\frac{dC}{dt} = \frac{\alpha_C R^{\eta^3}}{(\theta_R)^{\eta^3}+R^{\eta^3}} - \gamma_C C$$

$$\frac{dR}{dt} = \rho_R[LuxR]^2 A^2 - \gamma_R R$$

$$\frac{A_{x,y}}{dt} = \xi\big(A_{x-1,y} + A_{x+1,y} + A_{x,y-1} + A_{x,y+1} - 4A_{x,y}\big) - \gamma_A A$$

2.3 Negative auto-regulation

A constitutively expressed reporter driven by a $tetR$ regulated promoter typically exhibits a steep dose-response owing to the high cooperativity of $tetR$. However, the authors in [6] demonstrate that by using the principle of negative auto-regulation, wherein the expression of $tetR$ is regulated by a $tetR$ responsive promoter, one can achieve a reasonably linear dose-response. Based on the underlying mathematical structure of a negatively auto-regulated circuit, the authors provide a simple argument for the observed linear dose-response [6].

$$\frac{dx}{dt} = aF_x(x) - bxy - dx; \frac{dy}{dt} = C - bxy - fy; \frac{dz}{dt} = aF_z(x) - dz$$

where the variables x, y and z correspond to the free intracellular repressor, inducer and reporter concentration, respectively and C is a control parameter proportional to extracellular inducer concentration. At steady state, especially when dx and fy are negligible compared to bxy, we find that $z \propto C$ provided F_z and F_x are related via a linear transformation i.e., if the reporter and $tetR$ are driven from similar promoters. We also find that the above equations predict that the linearization is robust to parameter variations as z is independent of a or b. Variation analysis using the above equations also indicates that negative auto-regulation reduces the noise in reporter gene expression which the authors confirmed experimentally.

2.4 Positive-feedback linearization

The authors in [2] demonstrate that one can also achieve linearization by means of graded postive feedback. Specifically, the authors place a positive-feedback circuit on a low-copy number plasmid (LCP) and a 'shunt' component on a high-copy-number plasmid (LCP). The shunt on the HCP is intended to buffer away any excess transcription factors expressed from the LCP such that the concentration of transcription factors is not saturating at usual operating conditions. The key to linearization via positive feedback is the use of a LCP housing the positive-feedback circuit. By using a variable copy number plasmid (VCP) and by increasing the copy number of the VCP, the authors demonstrate switching from an analog response to a digital response, confirming the hypothesis. Mathematically, the positive-feedback circuit can be visualized via a simplified form [2] as below:

$$f(I_n) = a.\frac{(\frac{I_n}{b})^n}{1 + (\frac{I_n}{b})^n} + d$$

where I_n is the inducer concentration, n is the Hill coefficient, a is an amplification factor, d is the basal level of expression and $f()$ represents the output. For small values of $ln(1+x)$, $ln(1+x) \simeq \frac{x}{1+x}$ and hence $f(I_n) \simeq a.ln\big(1 + (\frac{I_n}{b})^n\big) + d$

2.5 Novel analog circuits

We now turn our attention to a couple of novel synthetic circuits that can implement analog computation. Recently, the authors in [7] present a mathematical model which demonstrates that allosteric proteins can respond to stimuli on a logarithmic scale. Beginning with the widely used MWC model of allostery, and limiting the ligand concentration range to be large enough to facilitate binding to the active conformation but not so large to allow binding to the inactive conformation,the authors demonstrate [7] that

$$\frac{da}{dt} = S(c, \varepsilon_0)\frac{d}{dt}\left(ln\frac{c}{K_A}\right)$$

where $S(.)$ represents a sensitivity function, a is the fraction of proteins in the active state and c is the concentration of the ligand. Although logarithmic response to inducer concentration is common in transcriptional circuits, the above framework combined with practical approaches towards engineering allostery [5] can help generate novel logarithmic computation at the protein level.

Another analog circuit we propose is a protein level ratio meter. One can potentially employ the concept of competitive binding to design a reporter gene that relays the relative abundance of competing transcription factors. Mathematically, the rate of production v of the reporter protein can be related to the ratio ρ of competing transcription factors TF and I as follows:

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

3. CONCLUSION

Analog synthetic gene circuits offer the advantage of implementing complex computation with relatively few biological components. However, identifying the appropriate biological components that can give rise to a specific underlying mathematical structure is often times challenging. In building novel analog circuits, a synthetic biologist can potentially explore building analog circuits that operate at the RNA or the protein level, or try molecular components that are relatively less characterized mathematically, such as Cas9 from *S. pyogenes*. Alternatively, circuit topologies that can combine multiple interacting components in a creative fashion can also give rise to novel computation.

4. REFERENCES

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