

Temperature Dependence of Biomolecular Circuit Designs

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Abstract—Quantifying performance of biomolecular circuit designs across different environmental conditions is a key step in assessing their robustness. It is generally unclear how robust this performance is to the important environmental variable of temperature. Here, we address this issue for a transcriptional negative feedback circuit design that can speed up the response time using a combination of simple computational methods and dynamic experimental measurements. We use a simple two-state model of gene expression to illustrate different ways in which temperature dependence of reaction rate parameters can propagate through to the functional output. Next, we extend this analysis to the response time of a transcriptional negative feedback circuit design. Finally, we present experimental results characterizing how response time of a negative transcriptional feedback circuit depends on temperature. These results help to develop framework for assessing how functional output of biomolecular circuit designs depend on temperature.

I. INTRODUCTION

Temperature is an important variable that can impact many natural and engineered systems. In particular, for engineering design, temperature-related specifications include ensuring that devices operate reliably across a reasonable range of temperatures as well as in products that can amplify or attenuate changes in temperature. As biomolecular circuits are designed from chemical reactions whose rates generally depend on temperature, circuit function may also depend on temperature (Fig. 1). Understanding this temperature dependence is an important challenge for synthetic biology, both to characterize the extent of temperatures for which circuits operate reliably as well as for the design of circuits with temperature-related function, and may also offer insights to the role of temperature in naturally occurring circuits.

Indeed, naturally occurring biomolecular circuits can implement a range of dynamic cellular responses that are robust to environmental disturbances. Replicating this feature has been a major driving force for design using biomolecular substrates. One example of designing a dynamic behavior is the construction of biomolecular oscillators through a combination of positive and negative feedback [1]–[5]. Period of oscillation in naturally occurring oscillators (for example [6]) can be robust to temperature. Recent work has focussed on characterizing the temperature dependence of oscillator designs [1]. Another example of a designing dynamic behavior is the demonstration of faster response time using negative transcriptional feedback [7]. This study developed

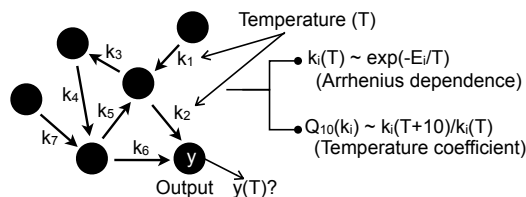


Fig. 1. Temperature dependence in a biomolecular circuit propagates from reaction rate parameters to functional output. Schematic illustration of a biomolecular circuit. Arrows represent biochemical interactions between different biomolecules. These are denoted with solid circles. The functional output (y) of this circuit depends on the reaction rate parameters (k 's). Temperature dependence of reaction rates can be characterized using an Arrhenius formalism or the temperature co-efficient Q_{10} . In the Arrhenius formalism, reaction rate k is represented as $k_0 \exp(-E/RT)$, where k_0 is a pre-exponential factor, E is activation energy, R is the universal gas constant, and T is temperature. The Q_{10} factor of a reaction rate at a given temperature T is defined as the ratio between its value at a temperature $T + 10$ and its value at temperature T . Typically, Q_{10} values are in the range 2–3 [13].

a method to measure this key parameter by relying on a remarkable property of the circuit that allowed it to be turned on in response to an inducer. In addition to the experimental demonstration that the response time can be sped up to a fraction of the cell cycle, which is the typical timescale of transcriptional response, this study developed a simple mathematical model to guide the design. In this model, the response time depends on the circuit parameters, including the promoter strength, promoter binding, and the cell cycle timescale. These elements can change with temperature and lead to different response times for different temperatures.

Interestingly, investigations have shown that the functional output in biomolecular circuits can be both robust to and sensitive to temperature. In the first case, the functional output may be independent of temperature over a range of temperatures. Such a flat temperature dependence is known for certain naturally occurring oscillation circuits [6] as well as, more recently, for chemotaxis in *E. coli* [8]. Contrastingly, in the second case, the functional output can also be sensitive to temperature, switching abruptly between two widely different values at a characteristic threshold value of temperature. An example of this is the detection of an increase in temperature by specific RNA molecules in bacterial cells [9]. Such temperature sensitivity is also observed for certain sex-determination systems [10] and harnessed for genetic studies [11] as well as in recombination technologies [12]. These two represent extreme scenarios of how the output of biological circuits depend on temperature. In general, how the function in biomolecular circuit designs depends on temperature is unclear.

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Here we use the response time speed-up due to a transcriptional negative feedback circuit design as a case study to investigate the temperature dependence of biomolecular circuit designs. To study this question, we use a combination of simple mathematical models and dynamic fluorescent measurements inside cells. With a two-state model that can be used to represent a wide range of biomolecular circuits from gene expression to signaling, we illustrate different ways through which temperature dependence can propagate from the reaction rate parameters to the functional output. Next, we computationally analyze how the response time of a transcriptional feedback circuit can depend on temperature, assuming the reaction rate formulas depend on temperature with a temperature coefficient (Q_{10}) in the range of 2–3. Finally, we perform experimental measurements to characterize how the response time depends on temperature. These results help to develop a framework to analyze temperature dependencies in biomolecular circuit designs.

II. ILLUSTRATIVE EXAMPLE

As an illustrative example for how temperature dependence can propagate through a biomolecular circuit, we first consider a two-state model. Such two-state models have been used to represent the behavior of different biomolecular processes including gene expression and covalent modification due to phosphorylation. Consider a two-state model in the context of gene expression. At its core, this consists of a promoter that can either have the RNA polymerase bound to it or not (Fig. 2A). We denote the state where the promoter is bound to the RNA polymerase as D_1 and the state where it is free of the RNA polymerase as D_0 . As the total amount of promoter is conserved, $D_0 + D_1 = \text{a constant}$, say D_T . We assume that the binding and unbinding reactions are first order and proceed at rates k_1 and k_2 , respectively. These are the reaction rate parameters. The output (y) is the fraction of promoter bound to the RNA polymerase. Using the standard mass action based kinetics, the rate of change of D_1 can be written as,

$$\frac{dD_1}{dt} = k_1 D_0 - k_2 D_1. \quad (1)$$

At equilibrium, this rate of change equals zero so that $k_1 D_0 = k_2 D_1$. The equilibrium output is (Fig. 2B, C),

$$y = \frac{D_1}{D_T} = \frac{k_1}{k_1 + k_2}. \quad (2)$$

The binding and unbinding reaction rate parameters may depend on temperature. In particular, we first assume this temperature dependence to be in the Arrhenius form, so that $k_1 = k_{1,0} \exp(-E_1/RT)$ and $k_2 = k_{2,0} \exp(-E_2/RT)$. Here, $k_{1,0}$ and $k_{2,0}$ are pre-exponential factors, E_1 and E_2 are activation energies, R is the universal gas constant, and T is temperature. Because of the nonlinear form of the output expression, it is unlikely that the output y will exhibit an Arrhenius temperature dependence. To examine this further, we considered two limiting cases (Fig. 2C). In the first limit $k_1 \ll k_2$, the output is $y = k_1/k_2$. In this limit, the output is also an Arrhenius function of temperature

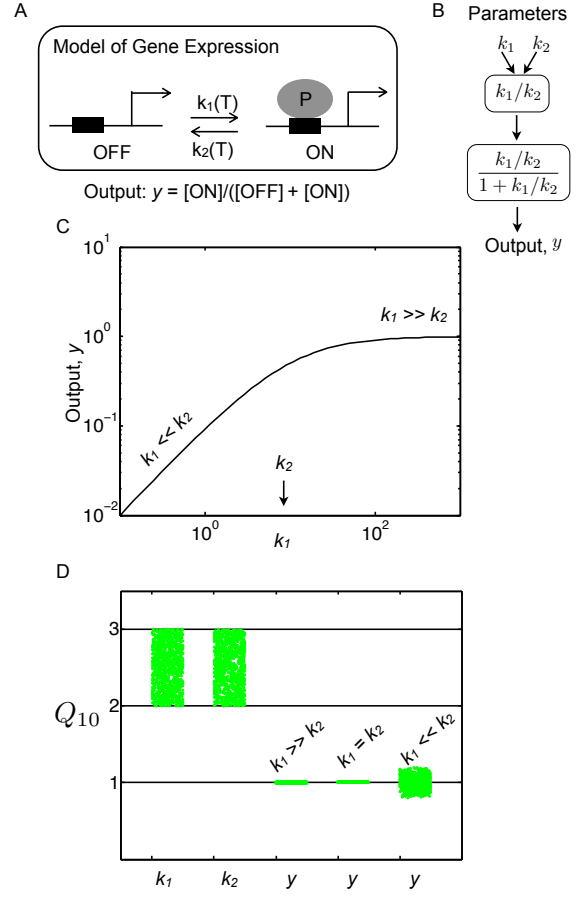


Fig. 2. Propagation of temperature dependence in a two-state model. A. Schematic illustration of a two-state model representation of gene expression. Black arrow on a black horizontal line denotes the promoter region. Black box represents the binding site of the RNA polymerase. Grey ellipse denotes a RNA polymerase molecule. B. Flowchart illustrates how reaction rate parameters combine to generate functional output. C. Solid line shows the equilibrium dependence of the output on the reaction rate parameter k_1 . D. Green dots illustrate the range of possible output Q_{10} values in the limits $k_1 \gg k_2$, $k_1 = k_2$, and $k_1 \ll k_2$. $k_2 = 10/\text{hr}$ is fixed in all cases and values of k_1 are chosen as $10^3/\text{hr}$, $10/\text{hr}$, and $10^{-1}/\text{hr}$, respectively. Horizontal spread around each value of is an arbitrary random number chosen to illustrate the distribution of green dots. Solid black lines in the background represent key Q_{10} values. Each parameter is randomly assigned a Q_{10} value between 2 and 3. A Q_{10} value of 1 for a quantity indicates that it is independent of temperature.

with a pre-exponential factor $k_{1,0}/k_{2,0}$ and energy $E_1 - E_2$. In the second limit $k_1 \gg k_2$, the output is $y = 1$. This value is independent of temperature. It can be assigned an Arrhenius dependence with zero energy, $E_y = 0$. However, for the entire space of parameters, there is no simple Arrhenius description of this process. Therefore, we note that the temperature dependence of this output can exhibit a non-Arrhenius temperature dependence even though the constituent reaction rate parameters exhibit an Arrhenius temperature dependence.

Next we perform the same exercise by characterizing temperature dependence of a reaction rate by its Q_{10} value. The Q_{10} factor of a reaction rate at a given temperature T is defined as the ratio between its value at a temperature $T + 10$

and its value at temperature T . We choose this to be in the range 2–3. We use the same limits as above to organize the temperature dependence of the output. In the first limit of $k_1 \ll k_2$, the output is $y = k_1/k_2$. As the Q_{10} 's of both reaction rates are in the range 2–3, the maximum value of the Q_{10} of the output is 1.5 and its minimum value is 0.66. In the second limit $k_1 \gg k_2$, the output is $y = 1$. The Q_{10} in this limit is also 1. To complete this picture, we randomly assigned a Q_{10} value in the range 2–3 for both k_1 and k_2 and calculated the Q_{10} of the output y for the cases $k_1 \ll k_2$, $k_1 = k_2$, and $k_1 \gg k_2$. Based on these simulations (Fig. 2D), we note the same point that the temperature dependence of output when characterized by its Q_{10} value can differ from that of the reaction rate parameters.

In the context of covalent modification due to phosphorylation, the two states in the model correspond to the phosphorylated and unphosphorylated forms. The interconversion between these two forms depends on rate constants that are temperature dependent. In a manner similar to above, it can be shown that the fraction of phosphorylated protein can exhibit a temperature dependence that is different from that exhibited by the reaction rate parameters. These results illustrate complex ways through which temperature dependence can propagate through a biomolecular circuit.

III. NEGATIVE TRANSCRIPTIONAL FEEDBACK

We repeated the above analysis for determining how temperature dependence of reaction rate parameters in a negative transcriptional feedback circuit propagates to its response time. For this, we introduce a simple model of transcriptional negative feedback, previously used to show that the response time using negative transcriptional feedback can be faster than the cell cycle timescale and that allows for obtaining an analytical expression for response time (Fig. 3A–B, [7]). The model consists of a protein X which negatively regulates its own expression. The rate of change of X can be mathematically expressed as,

$$\frac{dx}{dt} = \frac{\beta}{1 + x/k} - \alpha x. \quad (3)$$

Here, x is the concentration of the protein X . Negative regulation is modeled as a Hill function with coefficient equal to 1, a maximal production rate of β , and a DNA binding constant of k . The protein also dilutes as a cell grows and divides during the cell cycle process. This dilution is modeled as a first order process with rate constant α .

The response time of this circuit is defined as the fraction of cell cycle timescale ($\ln 2/\alpha$) that is required for the response to reach half its final value. To obtain an analytical expression for it, we first normalize Eqn. 3 using a dimensionless concentration $y = x/k$ and a dimensionless time $\tau = \alpha t$,

$$\frac{dy}{d\tau} = \frac{p}{1 + y} - y, \quad p = \frac{\beta}{\alpha k}. \quad (4)$$

The final value is reached when $dy/d\tau = 0$. This allows us to calculate the final value (denoted y_0) as the solution of

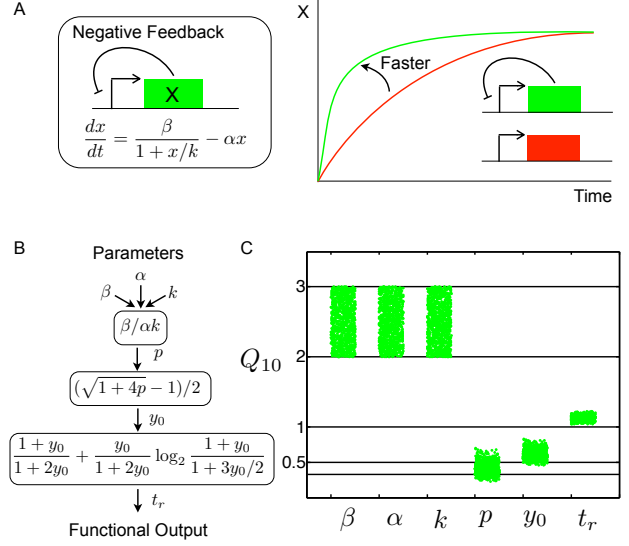


Fig. 3. Propagation of temperature dependence in a model of negative transcriptional feedback. A. Illustration of a biomolecular circuit with negative transcriptional feedback and its effect in speeding up the response time. Protein X is a transcription factor and represses its own production. B. Flowchart illustrates how reaction rate parameters combine to give the response time. C. Green dots illustrate the range of possible output Q_{10} values of the reaction rate parameters $\{\beta, k, \alpha\}$, the response time t_r , and the intermediary parameters y_0 and p . Parameters used in simulation were $\beta = 1000 \text{ nM/hr}$, $k = 10 \text{ nM}$, and $\alpha = 1/\text{hr}$. Horizontal spread around each value is an arbitrary random number chosen to illustrate the distribution of green dots. Solid black lines in the background represent key Q_{10} values. Each parameter is randomly assigned a Q_{10} value between 2 and 3. A Q_{10} value of 1 for a quantity indicates that it is independent of temperature. Q_{10} values of $1/2$ and $1/3$ are indicated as they represent the Q_{10} values of reciprocals of quantities whose Q_{10} is 2 and 3, respectively.

the equation $y^2 + y - p = 0$. This quadratic equation has two solutions, only one of which is positive. This positive solution is the required final value, $y_0 = \frac{\sqrt{1+4p}-1}{2}$. The time $t_{1/2}$ required to reach half the final value $y = y_0/2$ starting from $y = 0$ at $t = 0$ can be obtained by directly integrating Eqn. 4,

$$\begin{aligned} \int_0^{t_{1/2}} d\tau &= \int_0^{y_0/2} \frac{(1+y)dy}{p-y-y^2} \\ \Rightarrow t_{1/2} &= - \int_0^{y_0/2} \frac{(1+y)dy}{(y-y_0)(y+y_0+1)} \\ &= \frac{1+y_0}{1+2y_0} \ln 2 + \frac{y_0}{1+2y_0} \ln \frac{1+y_0}{1+3y_0/2} \end{aligned}$$

Due to the use of dimensionless time, $t_{1/2}$ is already normalized by a factor $1/\alpha$. To obtain an analytical expression for response time, $t_{1/2}$ needs to be additionally scaled by $\ln 2$. The analytical expression for the response time is,

$$t_r = \frac{1+y_0}{1+2y_0} + \frac{y_0}{1+2y_0} \log_2 \frac{1+y_0}{1+3y_0/2}. \quad (5)$$

Here, $y_0 = \frac{\sqrt{1+4p}-1}{2}$ and $p = \frac{\beta}{\alpha k}$.

The mapping from the reaction rate parameters $\{\beta, k, \alpha\}$ to the response time t_r is complex. As noted in the previous section, even simple expressions from parameters to output can result in complex temperature dependencies. To

understand the propagation of temperature dependence from reaction rate parameters to the output, we use simulations that randomly assigned a Q_{10} value in the range 2–3 for each of the parameters β , α , and k . These were then used to calculate the Q_{10} of the output t_r (Fig. 3C). Additionally, we also noted the Q_{10} of the intermediary parameters p and y_0 . These simulations show that the Q_{10} of the response time t_r is close to 1 even though the Q_{10} of parameters is in the range 2–3. Therefore, the response time t_r can be almost independent of temperature even if the reaction rate parameters depend on temperature.

IV. EXPERIMENTAL RESULTS

We performed experimental measurements to characterize the dependence of response time in a transcriptional negative feedback circuit on temperature. For this, we based our measurements on previously described materials and methods [7], and repeated the measurement at different temperatures — 25°C, 30°C, 32°C, 37°C, and 42°C. The strain used for measurement was an *E. coli* DH5 α strain containing the plasmid pZS*21-TetR-egfp. This plasmid encodes for a transcriptional negative feedback circuit with the fluorescent protein fusion TetR-egfp expressed from a P_{tet} promoter.

For measurement, this strain was first grown overnight in minimal media M9CA (Teknova) at the required temperature. Subsequently, the overnight culture was diluted 1:100 in the same media containing different levels of inducer aTc. Measurements were performed in black clear-bottomed 96-well plates (Perkin Elmer). Each well of this plate contained 200 μ l of cell culture with appropriate inducer level and was overlaid with 50 μ l of mineral oil (Sigma) to prevent evaporation. These plates were placed in a plate reader (Perkin Elmer Victor X3) to measure both fluorescence (excitation - 485 nm, emission - 535 nm) and optical density (OD) at 10 minute intervals for approximately 20 hours and at the required temperature. These measurements were taken in triplicate on each day, and were repeated for multiple days (three for all temperatures and two for 30°C).

Measured data were analyzed in MATLAB. From fluorescence and OD trajectory of each well, background of a well containing only media and no cells was subtracted. The OD reading gives an estimate of the number of cells at each time point and its behavior as a function of time provides an estimate of the rate of growth and division due to the cell cycle. This cell cycle timescale was estimated as the time taken for cells to grow from an OD of 0.02 to an OD of 0.04. At 42°C, cells do not grow, and these measurements were not considered further. Measurements with aberrant OD profiles were also discarded from analysis. For analysis of fluorescence traces, autofluorescence, estimated through the fluorescence of cells in DH5 α background (DH5 α Z1, [14]), was subtracted from all fluorescence readings. Fluorescence traces were smoothened using a moving average filter ('smooth' function in MATLAB with default options).

The key step in this method of measuring the response time is to determine the time at which the circuit transitions

to negative feedback behavior. This is determined based on the property that the difference in the fluorescence at this transition time and the final fluorescence depends only on the negative feedback circuit. This constant can then be determined through a plot of the final fluorescence vs aTc. We determined this constant through the y -intercept of a linear fit to the plot of final fluorescence vs aTc for the inducer concentrations {0, 1.56, 3.13, 6.25} (in ng/well). The time of transition is then the time at which the fluorescence is lower than the final fluorescence by this constant value. Fluorescence value above this constant and from the transition time to the end of growth (determined visually from corresponding OD trace) represent the dynamics due to negative feedback. Further, fluorescence values at each time were divided by the corresponding OD value to estimate fluorescence per cell. To obtain time in cell cycles, the time-axis was replaced with logarithm (to base-2) of OD at a given time to the OD at the transition time. Response time was counted as the time in which this trace reached half its maximal value.

The results of these measurements are shown in Fig. 4. The response time of 0.4 at 37°C is close to the value of 0.2 obtained previously [7]. We find that the response time is reasonably constant over the range 25°C–37°C. In this temperature range, we find that the cell cycle timescale changes, with a minimum at 30°C. Therefore, these measurements show that the response time of a transcriptional negative feedback circuit persists over a range of temperatures even though the important parameter of cell cycle timescale changes.

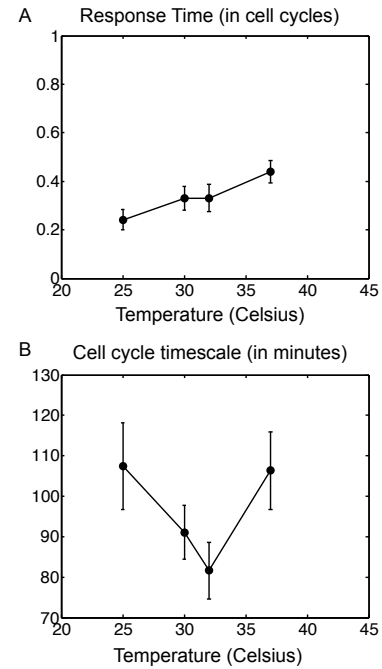


Fig. 4. Measurements of response time in transcriptional negative feedback circuit in the temperature range 25°C–37°C. Black dots represent the mean value of A. response time, and B. cell cycle timescale, for different temperatures. These measurements are for aTc concentration of 1.56 ng/well. Error bars are standard deviation over repeats.

V. CONCLUSIONS AND FUTURE WORK

Characterizing behavior of biomolecular circuit designs across different environmental contexts, such as different temperatures, is a key step in developing a systematic design process. Using a combination of mathematical models and dynamic experimental measurements, we present three results aimed at addressing this issue through a case study of a negative transcriptional feedback circuit design that can speed up the response time. First, we illustrate complex ways in which temperature dependence of reaction rate parameters can propagate through to the functional output through an example of a two-state model. Second, we computationally analyze the dependence of response time in a transcriptional negative feedback circuit on temperature. Third, we present experimental measurements aimed at characterizing how response time depends on temperature, finding that it is relatively independent of temperature. These results should help develop a framework to investigate effects of temperature in biomolecular circuit designs.

An interesting aspect of these results is the analog between instances where the output is independent of temperature and how robustness to temporal changes in reaction rate parameters is achieved. For example, the output can be independent of temperature in two limits of the two-state model. In the first limit ($k_1 \ll k_2$), the output is the ratio k_1/k_2 . If the activation energies for both these rates are equal ($E_1 = E_2$), then the output is independent of temperature. In fact, when the activation energies are equal, the output is independent of temperature for the entire parameter space. This independence stems from the fact that it is the ratio of reaction rate parameters that affects the output and the temperature dependence of the numerator and denominator in this ratio are pre-programmed to be identical. In the second limit ($k_1 \gg k_2$), the output is close to 1. This is also independent of temperature as the temperature dependence of the numerator through the parameter k_1 is counteracted by the temperature dependence of the denominator that is dominated by k_1 in this limit. As the sensitivity of the output to k_1 is reduced in this regime, this independence to temperature is at a cost of reduced gain. The first case where there is a pre-programmed cancellation of temperature dependencies is analogous to a feedforward mechanism. The second case where a parameter counteracts its own effect is analogous to a feedback mechanism. Such combinations of reaction rate parameters also appear in the expression for the response time of a negative transcriptional feedback circuit. Therefore, there appear to be parallels in instances where the output is independent of temperature with how robustness to temporal changes in biomolecular circuit parameters is implemented with the use of feedforward and feedback mechanisms.

An important task for future work is to develop methods to directly measure the temperature dependence of reaction rate parameters as well as to similarly characterize temperature dependence of other biomolecular circuit designs. In particular, cell-free systems [15] provide a potentially convenient

platform to directly measure the temperature dependence of reaction rate parameters as well as to independently verify the measurements presented here. Benchmark case studies for similar characterization include the pulse generation property of the incoherent feedforward loop [16] and the periodic waveforms of biomolecular oscillators [1], [2]. In fact, preliminary computational results in feedforward loop models suggest that the properties related to time should depend on temperature whereas those related to amplitude can be independent of temperature. Together, these investigations should provide a comprehensive framework for understanding temperature effects in biomolecular circuit designs.

Characterizing temperature dependence of biomolecular circuit designs helps to assess their functional robustness to temperature. Results presented here address this for the case of a negative transcriptional feedback circuit. Additionally, this should help in two complementary goals. One, to provide a platform for developing a toolbox of biomolecular circuits with temperature-related function. Two, for insights into the role of temperature in naturally occurring biomolecular systems. Considerations of different ways that ensure functional robustness to temperature presented here have direct implications for both these goals.

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