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Performance Metrics for a Biomolecular Step Response

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***Abstract*— Establishing performance metrics is a key part of a systematic design process. In particular, specifying metrics useful for quantifying performance in the ongoing efforts towards biomolecular circuit design is an important problem. Here we address this issue for the design of a fast biomolecular step response that is uniform across different cells and widely different environmental conditions using a combination of simple mathematical models and experimental measurements using single-cell time-lapse microscopy. We evaluate two metrics, the difference of the step response from an ideal step and the relative difference between multiple realizations of the step response, that can provide a single number to measure performance. We use a model of protein production-degradation to show that these performance metrics correlate with response features of speed and noise. Finally, we work through an experimental methodology to estimate these metrics for step responses that have been acquired for inducible protein expression circuits in *E. coli*. These metrics will be useful to evaluate biomolecular step responses, as well as for setting similar performance measures for other design goals.**

I. INTRODUCTION

Significant progress has been made in the design of biomolecular circuits. These include demonstrations of circuits that can respond quickly [1], oscillate [2], [3], and switch from one state to another [4]. There are two major challenges for the next stage of biomolecular circuit design. The first challenge is to ensure robust operation of these circuits in different environmental conditions. The second challenge is to implement circuits with a larger number of components by connecting existing circuits with smaller component numbers [5]. In general, these challenges are interrelated. Robust circuit performance should help in connecting circuits with one another, and larger circuits may exhibit robust circuit performance. A systematic design process that focuses on characterizing circuit performance and developing a specification-oriented methodology should facilitate solutions to these challenges. An important part of this process is to define performance metrics for biological circuits that are useful for bioengineers.

Quantitative performance measures are widely used in engineering design. For example, a typical controller design might start with specifications for sufficient gain and phase margins to ensure stability. These are then used to determine the desired controller parameters. If the controller is to be implemented electronically, the controller parameters are matched against the performance specifications of the electronic device listed on its data sheet. Precise knowledge of the quantitative capabilities of the device and the ability to compare it with the requirements helps in a systematic design process. In accordance with such a design process, there have been efforts to catalog properties of biomolecular parts and devices [6]. More recently, as part of a combined effort aimed at a critical assessment of biomolecular circuit designs (CAGEN, [7]), there have been steps towards a specification oriented-design.

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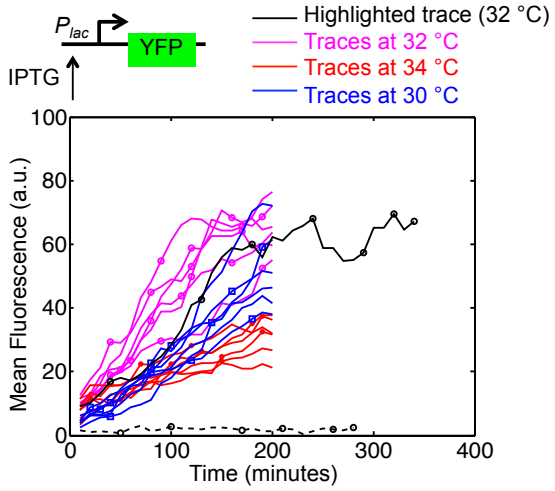

Fig. 1. Step responses of an inducible protein expression circuit in single cells. Schematic illustrates the biomolecular circuit in which a fluorescent protein YFP can be induced by a chemical IPTG. Solid black line is an example of a step response from a single cell. Red, magenta and blue lines represent step responses in other cells at temperatures 34°C, 32°C, and 30°C, respectively. Symbols like circles, squares, and dots on each trace denote the time of cell division. Dashed black line is a trajectory from a single cell that is not induced. All traces show mean single-cell fluorescence values above camera background. The concentration of IPTG used for induction is 1mM. The time of induction is ≈ -10 min.

Consider the goal of designing a biomolecular circuit that responds quickly to a step change in input signal in a way that is uniform across different cells and environmental conditions. A step response for an inducible protein expression circuit in *E. coli* [8] for a single cell is highlighted in Fig. 1 (solid black line). The other responses are for different cells at either the same temperature as the highlighted trace or at slightly different ones (colored lines, Fig. 1). Here, temperature is used to illustrate the possible effect of different environmental conditions. These measurements underline three aspects that are typical in biomolecular step responses — a timescale that determines when the response reaches its equilibrium, an equilibrium amplitude sufficiently distinct from a basal level, and variability that can exist in responses from different cells and across conditions. Accounting for

these features is important in generating a single number that can be used to quantify the performance of a biomolecular step response.

Here we ask the question of how to generate a single number that can measure performance for a biomolecular step response. We address this issue using simple computational models of biomolecular circuits and experimental single-cell fluorescence time-lapse microscopy measurements acquired towards establishing baseline protocols for the CAGEN assessment [7]. First, we state two metrics, the difference of the step response from an ideal step and the relative difference among multiple realizations of a step response, that can generate a single number for a transient response. Second, we use a simple computational model of protein production-degradation to show that these performance metrics correlate with the properties of fast and uniform response. Third, we work through a sequence of steps to estimate these performance metrics experimentally for simple inducible protein expression circuits from *E. coli*. Our results should be useful to bioengineers in establishing performance metrics for a biomolecular step response.

II. PERFORMANCE METRICS

We first revisit the transient performance specifications for a step response in the context of an electronic amplifier [9]. These specifications capture how the output responds to a step increase in input voltage. There are three key measurements that are typically used for this purpose (Fig. 2). One, the settling time, or the time it takes for the response to reach an equilibrium. This can be estimated as the time it takes to go from 10% of the final value to the time after which the response stays within 10% of this final response. Two, the rise time, or the speed of the response. This can be estimated as the time it takes to go from 10% of the final value to 90% of its final value. Three, the overshoot, or the extent of deviation of the amplitude from an ideal step. This can be estimated as the amplitude of the maximum value of the response relative to the final value.

By replacing the voltage waveform with that of protein concentration, the transient response specifications discussed above can be transferred to a biomolecular step response. These provide a set of measurements that quantify the speed of the response, an important property of a step response. The second feature of a biomolecular step response that needs to be quantified is its amplitude relative to the basal level. This is important as expression and/ or activity of proteins has a basal non-zero level even when they are turned off. One possibility to ensure sufficient amplitude is to require that the equilibrium value in response to a step is at least 10 times the basal level. The third feature of the response that needs to be quantified is the extent to which it is uniform across different cells or widely varying conditions. Typical measurements of variability in biology are based on the ratio of the standard deviation of the equilibrium amplitude among cells to the mean value [10]. Together, the transient response measurements, fold-change upon induction, and equilibrium noise provide a set of individual measurements that can be used to quantify the step response.

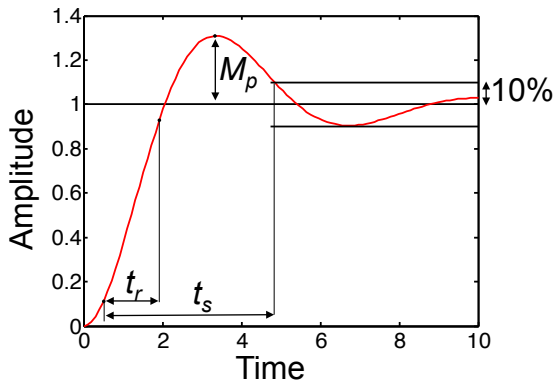

Fig. 2. Transient specifications of a step response. t_s, t_r, and M_p denote the settling time, rise time and maximum overshoot of a step response respectively.

Further, we note how normalizing these responses with their characteristic scale is an important step in contextualizing the design. This is because these designs may operate in diverse biological contexts, including different species, different genetic backgrounds within a species, and widely different environmental conditions for the same genetic background. In particular, the cell cycle is the characteristic timescale in most biological contexts, and normalizing time by cell cycle can be used to qualify the temporal specifications. Similarly, the concentration of a characteristic molecular species, such as a housekeeping sigma factor, can be used as a normalizing factor for amplitude. This is frequently used in various biological assays like blots and gels.

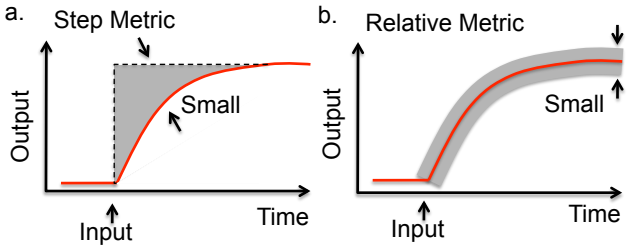

Fig. 3. Schematic illustrating the performance metrics. (a) Red line indicates a step response. Dashed black line indicates an ideal step. Grey region indicates the area calculated in the step metric. (b) Red line indicates a reference trace from a step response. Grey region indicates the possible spread due to multiple realizations of the step response. This is also the area calculated in the relative metric.

Based on how these individual specifications capture different aspects of the transient response, we state a performance metric that can generate a single number for each response. We base this performance metric on the difference between the transient response and an ideal step response (Fig. 3a.). Mathematically, this can be expressed as,

$$S = \frac{\int_{T_1}^{T_2} (y(t) - M)^2 dt}{M^2}.$$

Here, $y(t)$ is the measured response with equilibrium amplitude M, T_1 is a time close to the initiation of the response, for example, when the response is 10% of its final value, and T_2 is the settling time. One way of checking that the response reaches an equilibrium value M is to specify that the response is within 10% of its equilibrium value for an additional time $T_2 - T_1$ after T_2. A smaller value of this step metric indicates a better performance. As the value should decrease for a faster response, this performance metric should account for the important requirement of response speed.

Due to the variability associated with a biomolecular step response, there might be multiple realizations of each response. To compute a performance metric for this case, one of these traces can be chosen as a reference trace to determine the quantities M, T_1 and T_2. The step metric can then be computed in a worst-case fashion, as the maximum of the difference between an ideal step and each response,

$$S = \max_j \frac{\int_{T_1}^{T_2} (y_j(t) - M)^2 dt}{M^2}, j = 1, 2, 3 \ldots N.$$

Here, $y_j(t)$ are the multiple realizations. This performance metric should be able to penalize larger variability, as the worst-case value of a variable response will be generally higher than that of a less variable response. In this way, the metric should account for the variability in responses across different conditions.

An alternative measure of the performance is the relative variation among the traces (Fig. 3b.). This is another performance metric that can generate a single number for a response. Mathematically, this can be expressed as,

$$S_r = \max_j \frac{\int_{T_1}^{T_2} (y_j(t) - r(t))^2 dt}{M^2}, j = 1, 2, 3 \ldots N.$$

Here, $y_j(t)$ are the multiple realizations, $r(t)$ is a reference trace from among them which is used to obtain an equilibrium amplitude M, a time T_1 close to the initiation of the response, and a settling time T_2. This relative metric should decrease if all realizations are close to the reference trace. In other words, performance should improve if the responses are uniform.

III. SIMULATION RESULTS

To further explore the correlation between the step metric and speed, we used a standard model of a simple protein production-degradation circuit. In this model, the total level of a protein X depends on the balance between its production, modeled as a zero-order process that changes from a basal rate of α_0 to α_1 upon induction, and its dilution due to cell growth, modeled as first-order processes with rate constant γ. As a result of these interactions, the dynamics of the protein concentration X are,

$$\frac{dX}{dt} = \alpha - \gamma X,$$

where $\alpha = \alpha_0$ before induction and $\alpha = \alpha_1$ after induction. This differential equation can be analytically solved as,

$$X(t) = \frac{\alpha}{\gamma}(1 - \exp(-\gamma t)).$$

As the equilibrium value of the response is α/γ, the fold-change upon induction is α_1/α_0. A fold-change in excess of a factor of 10 should ensure sufficient increase in amplitude upon induction. Similarly, the speed of this response can be estimated from the analytic solution to be proportional to $1/\gamma$. Finally, the step metric for this trace can be calculated as,

$$S = \int_{T_1}^{T_2} \exp(-2\gamma t) dt = \frac{0.4}{\gamma}.$$

Here, $M = \alpha_1/\gamma$ is the equilibrium amplitude after induction, T_1 is the time when the response is 10% of its final value, and T_2 is the settling time. This shows that the step metric is directly proportional to the speed. As the response speeds up, this performance metric decreases, indicating a better response.

To evaluate the step metric for a set of variable responses, we generated multiple realizations of the step responses for this model (Fig. 4). These realizations were generated from stochastic simulations performed using the standard software package BioNets [11]. The performance score generated using these traces is 0.63 ($N = 15$). Recalculating the performance score for another set of stochastic traces ($= 0.63$, $N = 15$) or for a larger set of traces ($= 0.64$, $N = 100$) yields a similar value.

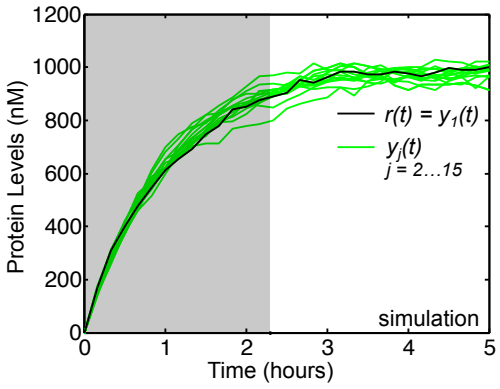

Fig. 4. Trajectories of the protein production-degradation process generated using stochastic simulations. Black trace is the reference trace, arbitrarily picked as the first trace, and green traces are the other 14 traces. All traces are sampled at a 10 minute resolution. Shaded area indicates the time duration ($T_1 = 0$, $T_2 \approx \ln(10)/\gamma$ hr) over which the performance score integral is evaluated. The model parameters for this calculation are $\alpha_1 = 1000$ nM/hr, $\alpha_0 = 1$ nM/hr, $\gamma = 1$ /hr.

Next, we used these stochastic simulations to verify that the step metric correlates with circuit properties of speed and noise in accordance with intuitive expectations. Both speed and noise can be analytically estimated for this model. As shown above, the response speed is proportional to $1/\gamma$. Similarly, using standard methods [12], the equilibrium noise can be shown to be $1/\sqrt{\alpha/\gamma}$. To compare the performance metric with these properties, we performed two additional set of simulations. First, the noise was increased for a fixed speed, by varying only α and not γ. Second, the speed was

increased at a fixed noise, by varying γ and changing α so that α/γ is fixed. For each value of noise and speed in these cases, two sets of 15 trajectories were simulated. The performance score was calculated as outlined above. In addition, for comparison with a larger number of trajectories, the performance score was also calculated for an additional set of 100 trajectories. For all sets of trajectories, we find that the performance score improves if noise decreases or if response speeds up (Fig. 5). The relation between the performance score and the response speed is especially strong.

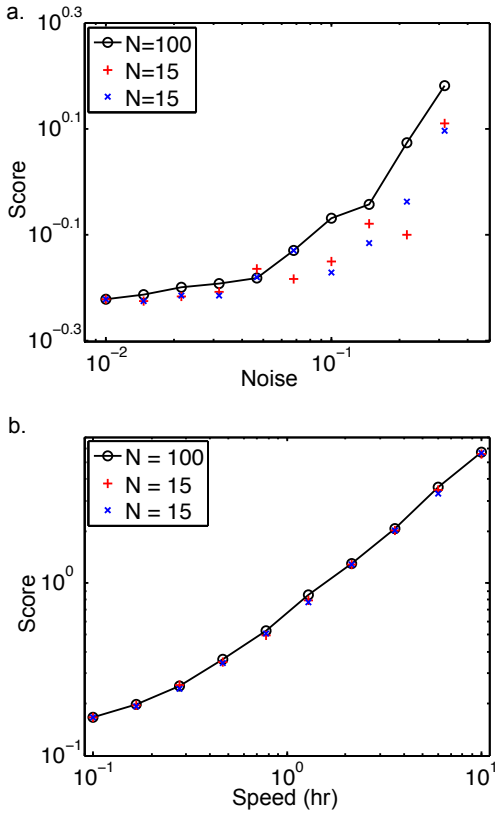

Fig. 5. Performance improves as noise reduces or response speeds up. Performance scores using the step metric are calculated as (a) noise is varied for fixed speed and, (b) speed is varied for fixed noise. Red crosses and blue circles are the performance scores for two sets of 15 trajectories. Black line connects points representing performance scores for a set of 100 trajectories, and are similar to the red crosses and blue circles. To change noise levels for fixed speed in (a), the values of α_1 are varied logarithmically from 10 nM/hr to 10^4 nM/hr, for fixed γ. To change speed values for fixed noise in (b), the values of γ are varied logarithmically from 0.1 /hr to 10 /hr and α_1 values are adjusted so that the ratio α_1/γ is fixed (= 1000 nM). The integrals are performed from $T_1 = 0$ to $T_2 \approx \ln(10)/\gamma$ hr.

We repeated these simulations to evaluate the relative metric. For multiple realizations of a step response, the performance metric for these traces is 0.005 ($N = 15$). A recalculation of this performance score for another set of traces (= 0.012, $N = 15$) or for a larger set of traces (= 0.008, $N = 100$) yields a similar value. Finally, comparing this performance metric with the circuit properties of speed and noise showed that the perfrmance score improves if noise decreases or if response speeds up (Fig. 6).

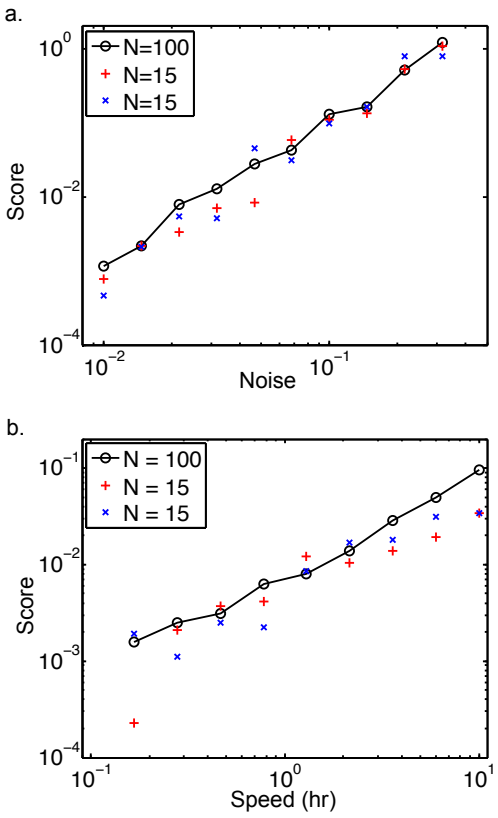

Fig. 6. Performance improves as noise reduces or response speeds up. Performance scores for the relative metric are calculated as (a) noise is varied for fixed speed and, (b) speed is varied for fixed noise. Red crosses and blue circles are the performance scores for two sets of 15 trajectories. Black line connects points representing performance scores for a set of 100 trajectories, and are similar to the red crosses and blue circles. To change noise levels for fixed speed in (a), the values of α_1 are varied logarithmically from 10 nM/hr to 10^4 nM/hr, for fixed γ. To change speed values for fixed noise in (b), the values of γ are varied logarithmically from 0.1 /hr to 10 /hr and α_1 values are adjusted so that the ratio α_1/γ is fixed (= 1000 nM). The integrals are performed from $T_1 = 0$ to $T_2 \approx \ln(10)/\gamma$ hr.

Together, these simulation results are in accordance with intuitive expectations of how the scores should vary relative to properties like speed and noise. It also underlines the utility of these performance metrics in generating a single number to capture the required specifications.

IV. EXPERIMENTAL RESULTS

As the next step, we worked through the task of estimating these performance metrics from experimental measurements. These measurements are from simple protein expression circuits in *E. coli*, where expression of proteins can be initiated with the addition of an inducer.

We measured the step response in single cells based on circuits and methodology used previously [8], [13]. Both circuits are encoded on plasmids that propagate in the *E. coli* strain background MG1655Z1. In these circuits, the addition of the inducer IPTG increases the expression of proteins, a yellow fluorecent protein (YFP, from plasmid pZS2-123, Fig. 1) and YFP fused to another protein CI (CI-YFP, from plasmid pNS2-σVL, Fig. 7). Both types of constructs, expression of YFP from the promoter region

of genes, which initiates protein production, and fusion of YFP to another protein, are widely used to report for the dynamics of protein concentrations in growing cells. Protein fusions have the advantage of reporting for specific protein activity, for example spatial localization, provided that the fusion itself does not distort the functionality of the native protein. Both types of reporters use the property that proteins such as YFP emit fluorescence when excited at their characteristic wavelengths. The dynamics of the process under measurement are also filtered by the reporter dynamics, such as the maturation time or the time it takes for a fluorecent protein to fluoresce after excitation.

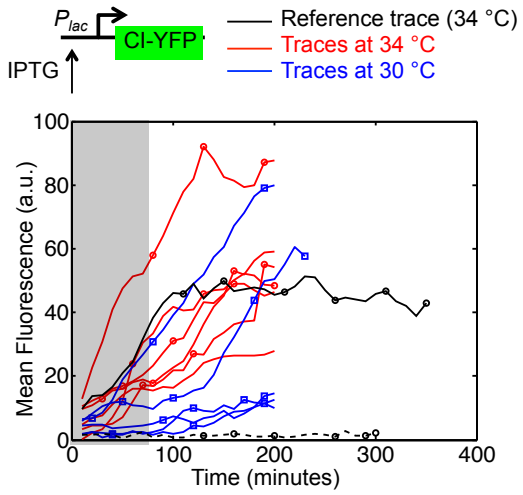

Fig. 7. Single-cell dynamics of an inducible protein expression circuit. Schematic illustrates a biomolecular circuit where the fluorescent protein CI-YFP can be induced in response to the chemical IPTG. Solid black line represents the reference trajectory. Dashed black line is a trajectory in the absence of induction. Red and blue colors represent the temperatures 34°C and 30°C, respectively. Symbols like circles, squares, and dots on each trace denote the time of cell division. All traces show mean single-cell fluorescence values above camera background. The time of induction is ≈ -10 min. The IPTG concentration for induction is 10 μM. Shaded region indicates the time duration over which the integral is evaluated.

For the measurement, cells were first grown overnight in selective LB media at 37° C. The overnight culture was diluted $1:100$ in MGC (M9 minimal media containing 0.2% Glycerol, 0.01% Casamino acids, 0.15 μ g/ml Biotin, and 1.5μ M Thiamine), and grown at 32°C for 3 hours. 0.5 μl of this culture was placed on semi-solid pads made out of MGC media containing 1.5% Low melting point Agarose (Omni) and the required amount of IPTG. These pads were flipped onto a glass-bottom dish (Wilco), sealed with parafilm, and then placed under the microscope. Induction of fluorescence was imaged every 10 minutes and at the required temperature using an automated microscopy setup (Nikon Eclipse-Ti inverted microscope with perfect focus, ASI motorized stage, Photometrix Coolsnap HQ2 camera, Sutter Lambda LS Xenon Arc lamp, and controlled using MetaMorph software). The resulting experimental data is in the form of a time-sequence of images. Processing these images to estimate fluorescence in each cell and tracking the change in fluorescence over time was performed using custom image processing software. To mimic variability in the responses due to different conditions, we performed measurements at different temperatures in the neighborhood of the nominal temperature of 32°C used previously [13].

For the first circuit, the reference trace reaches its equilibrium value of ≈ 60 a.u. (in arbitrary units) at $T = 160$ minutes (Fig. 1, black line). In the absence of induction, the equilibrium value for a trace is ≈ 2 a.u. Therefore, the reference trace exhibits a greater than ten-fold induction. The time after which this response stays approximately within 10% of this equilibrium value is $T_2 = 150$ minutes. Further, our measurements indicate that the response stays in this range for another duration of T_2 minutes, suggesting that this response settles at this equilibrium value. The other traces also show an increase in fluorescence in response to induction. Using the values of these traces in the time duration $T_1 = 0$ to $T_2 = 150$ minutes, we estimate the performance metrics for this dataset to be $S = 1.45$ for the step metric and $S_r = 0.29$ for the relative metric.

Similarly, the reference trace for the second circuit reaches its equilibrium value of ≈ 45 a.u. at $T = 100$ minutes (Fig. 7, black line). In the absence of induction, the equilibrium value of a trace is ≈ 1 a.u. Therefore, this reference trace exhibits a greater than ten-fold induction. This trace stays approximately within 10% of this equilibrium value after $T_2 = 80$ minutes. Further, in our measurements this response stays in this range for another duration of T_2 minutes, suggesting that this response settles at this equilibrium value. The other traces also show an increase in fluorescence in response to induction and their values in the duration $T_1 = 0$ to $T_2 = 80$ minutes are used to estimate the performance metric. The performance metrics for this dataset are estimated to be $S = 1.22$ for the step metric and $S_r = 0.30$ for the relative metric.

We note that these performance scores are largely similar. This is to be expected as even though one of the datasets appears more variable than the other, it is also faster. Therefore, these features balance each other and give rise to similar score. Together, these measurements establish a sequence of steps that can be used to estimate the performance metrics.

V. CONCLUSIONS AND FUTURE WORK

Defining performance metrics is an important step for the systematic design of biomolecular circuits. Here we address this for the design of a step response that is desired to be quick, of sufficient amplitude, and uniform across different cells and widely different environmental conditions through a combination of simple computational models of biomolecular circuits and experimental measurements with single-cell time-lapse fluorescence microscopy. First, we state two metrics, difference between the step response from an ideal step and the relative difference between multiple realizations of a step response, that can generate a single number for the performance of a circuit response. Second, we use a mathematical model of a protein production-degradation circuit to show that these performance metrics correlate with

circuit properties of speed and noise. Third, we use simple inducible protein expression circuits in *E. coli* to establish a sequence of steps to estimate these performance metrics experimentally. These should be useful in establishing a performance metric for biomolecular step responses.

An interesting feature of these specifications how normalization enables comparison across species, genetic backgrounds, and environmental conditions. In fact, this is analogous to the inherent normalization widely prevalent in control theory and engineering. For example, in measuring the gain of a system, the output amplitude is normalized by that of the input. Similarly, phase measurements automatically report the output timescale in terms of the input timescale. As the characteristic scales of amplitude and time may vary according to the biological contexts, normalization can be useful for biomolecular circuit design.

An important challenge for the future is to set performance specifications and metrics for other design goals, including for oscillators, switches, and spatial patterns. We expect that normalizing by the characteristic scales of time, space, and/or amplitude will be relevant for the specifications in these goals as well. In particular, the key specifications for an oscillator include its period and the peak amplitude, both of which can be normalized by the characteristic timescale and amplitude of the biological context. For an oscillator with sufficient peak amplitude, its period is typically the most important specification. Therefore, the difference between a thresholded version of the oscillation and a square wave of the specified period is one way of generating a single number that can be used as a performance metric. An alternative metric for performance is to measure the relative difference between multiple realizations of the oscillation responses.

A systematic design process for biomolecular circuits will facilitate applications in agriculture, in medicine, and in generating clean energy. Importantly, a comprehensive characterization of performance may also ensure operational safety, an important requirement for complex systems, and especially so for those constructed from biological substrates. Future work should reveal if the success of this design methodology in traditional engineering design has a parallel in the design of biomolecular circuits.

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