



# Real-Time Optogenetics System for Controlling Gene Expression Using a Model-Based Design

Guy Soffer, James M. Perry, and Steve C. C. Shih\*



ABSTRACT: Optimization of engineered biological systems requires precise control over the rates and timing of gene expression. Optogenetics is used to dynamically control gene expression as an alternative to conventional chemical-based methods since it provides a more convenient interface between digital control software and microbial culture. Here, we describe the construction of a real-time optogenetics platform, which performs closed-loop control over the CcaR-CcaS two-plasmid system in Escherichia coli. We showed the first model-based design approach by constructing a nonlinear representation of the CcaR-CcaS system, tuned the model through open-loop experimentation to capture the experimental behavior, and applied the model in silico to inform the necessary changes to build a closed-loop optogenetic control system. Our system periodically induces and represses the CcaR-CcaS system while recording optical density



and fluorescence using image processing techniques. We highlight the facile nature of constructing our system and how our modelbased design approach will potentially be used to model other systems requiring closed-loop optogenetic control.

core problem throughout synthetic biology is control A over gene expression. Over the years, there have been numerous works presenting standardized parts for modulating gene expression in various model organisms.<sup>1-6</sup> Tools like these are crucial to titrate the stoichiometric balance of target biochemical reactions and to limit toxicity within a cell. The control over the timing of gene expression is another important aspect that plays a crucial role in biochemical reactions. Applications such as chemical<sup>7</sup> or protein production<sup>8</sup> are improved further by decoupling biomass accumulation from production by externally toggling the expression of target genes. Dynamic control over the timing of cellular events is also very useful for discovering new biological processes.<sup>5</sup> Typically, dynamic gene control uses chemically inducible systems such as the lac operon in Escherichia coli<sup>10,11</sup> or galactose induction in Saccharomyces cerevisiae.<sup>7,12</sup> Despite widespread use, chemical induction suffers from several limitations. Chemical inducers inherently change the biochemistry of a system, potentially leading to crosstalk<sup>13</sup> and toxicity,<sup>14</sup> while the concentration will vary over time due to break-down.<sup>15</sup> There are also nucleic acid or CRISPRi regulators that have emerged as powerful tools to operate at the transcriptional or translational levels to control gene expression.  $^{16-20}$  These regulatory circuits have been used to further optimize metabolic pathways<sup>21,22</sup> or to enhance capabilities in precision medicine.<sup>23</sup>

A powerful method for controlling gene expression is optogenetics, which uses light to influence expression at the level of transcription.<sup>24,25</sup> Optogenetic switches are sourced from photodependent systems in bacteria,<sup>26,27</sup> plants,<sup>28,29</sup> and fungi<sup>30</sup> to name a few. In contrast to chemical induction, optogenetics offers precise time-varying modes of control since light can be instantaneously added or removed from a system.<sup>9</sup> Furthermore, automated light delivery can be achieved with minimal electronics and computer setup.<sup>31</sup> As a result, there has been a sharp increase in the number of recent optogenetic applications, particularly in the areas of closed-loop gene control.<sup>32–39</sup> With optogenetics, closed-loop control is achieved by modulating the light intensity and tuning a closed-loop controller using a model of a target system.<sup>9,40-47</sup> The most straightforward method to develop a closed-loop controller is to use a linearized model of a target system. Since optogenetics systems (e.g., CcaS-CcaR or CcaSR) are inherently nonlinear,<sup>25</sup> manually tuning a controller using a linear model to obtain estimates of the gains can be cumbersome and requires frequent trial-and-error experimentation. Starting with an accurate nonlinear model, rate parameters such as transcription and translation, protein degradation, and cell growth can be determined, providing accurate in silico predictions of the system's behavior.

Received: October 30, 2020 Accepted: January 25, 2021 Published: February 5, 2021



Recent optogenetics systems have not found widespread adoption due to various limitations. First, many of these setups are only capable of controlling expression in one sample.<sup>37,4</sup> This is not ideal if there is a desire to simultaneously test multiple conditions such as gene expression rate targets. 31,38,48 Typically, these works make use of custom light plate apparatuses to deliver two or more distinct wavelengths. Although these systems can be easily reproduced, they are not capable of both real-time measurement of optical density and fluorescence, as well as control over gene expression, unless integrated with a separate chemostat system.<sup>41</sup> In addition, works presenting closed-loop control<sup>9,41</sup> use trial-and-error experimentation to tune controllers and do not supply mathematical justification for their design choices, adding an unnecessary amount of time and uncertainty to executing the experiments. Given these limitations, we have developed a concise method for closed-loop control over gene expression using optogenetics.

We present an easy-to-construct optogenetics platform (which we call the "RT-OGENE" or "Real-Time Optical GENEtics" system) consisting of a camera with a zoom lens, 3D-printed parts, a heating element, and a light-emitting diode (LED) matrix circuit board fabricated in-house. Our system regulates the CcaSR gene network in E. coli while measuring both optical density and fluorescence. The integration of camera vision to measure samples greatly simplifies the setup and significantly reduces equipment cost when building an optogenetic system. We represent the CcaSR gene network (i.e., the plant-the transfer function between the input and output of the system) using a set of nonlinear differential equations with rate parameters established from open-loop experimentation. Next, we mathematically showed the sufficiency of a PID controller and comprehensively described in silico techniques to tune the controller, avoiding a lengthy trial-and-error process requiring long culturing periods. Finally, we show the capability of controlling gene expression in multiple bacterial samples cultured in a well plate and microfluidic formats under closed loop to verify the modelbased design and "hardware-in-the-loop" approaches.

## EXPERIMENTAL SECTION

Model-Based Design of the CcaSR-V2 Model. As shown in Figure S9, we implemented a model-based design (MBD)<sup>49</sup> approach to develop a controller for gene expression.<sup>50</sup> Briefly, the mathematical model represented the biological system (i.e., the plant) and was approximated to be a linear-time-invariant (LTI). Following the plant design, the entire system (PID controller, feedback, and plant) was modeled in the digital z-domain using two methods: the CasSR model was transformed using the zero-order-hold (ZOH) method and the controller, differentiator, and the lowpass-filter were transformed using Euler's backward method (Figure S11A). The sampling time for the discrete conversion was chosen to be 30 min. This time frame was set for two main reasons: (1) to minimize the cross-activation of the promoter by the GFP-signal itself and (2) to meet the minimum necessary sampling demand for the system's dynamics. A root locus method was used to tune the controller for stability using MATLAB Simulink Control System toolbox to convert the continuous time controller to discrete time controller and to perform the root locus analysis (Figure S10). For a description of the comprehensive model (time-invariant and variant

models) of the CcaSR system, see the Supporting Information and Tables S1–S4.

**Open-Loop Experiments.** Prior to the experiment, DH5 $\alpha$ co-transformed with pSR58.6 and pSR43.6r was diluted to 1:10 (to  $OD_{600} = \sim 0.3$ ) using LB supplemented with 1:1000  $(1 \ \mu g/mL)$  spectinomycin and 1:1000  $(1 \ \mu g/mL)$  chloramphenicol. The optogenetic setup was connected to a PC by a USB connection and to a 12 and 5 V power supply to drive the LEDs and the heating element. One hundred microliters of the cells were aliquoted into the well plate and incubated at 37 °C. Twenty microliters of silicon oil were loaded into each well to reduce evaporation of the samples. During the experiment, each well was exposed to different ratios of green-to-red light illumination conditions. Three open-loop experiments were performed using three different illumination schemes: (1) constant, (2) pulse, and (3) random illumination. For the constant illumination scheme, the wells were exposed to different ratios (0, 30, 45%) of green-to-red light for 6 h. For the pulse illumination scheme, cultures were exposed to a 40% green-to-red light ratio for 5 h. For the random illumination scheme, cultures were exposed to a range of ratios (0-90%) of green-to-red light ratio for 16 h. For all illumination schemes, cultures were initially exposed to red light for 1 h. Fluorescence and OD<sub>600</sub> values were measured periodically every 30 min and obtained using image-based techniques (as described in the light calibration section in the Supporting Information). All fluorescence measurements were initiated by exciting the samples using the blue LED set at 20% excitation power. The fluorescence and OD<sub>600</sub> values were periodically obtained by image-based techniques (see the Light delivery system, operation, and calibration in the Supporting Information) using an in-house Python program and recorded into a log file.

For open-loop microfluidic experiments, the media used in the device were identical to those used for the well plate (see above). The device was mounted on the plate holder inside the optogenetic setup and incubated at 37 °C for 21 h. At 0 h, the device was loaded with co-transformed CcaSR bacterial cells (at an OD<sub>600</sub> of 0.35). Each microfluidic well was exposed to one of the ratios of green-to-red light -10, 20, 40, and 60%. Fluorescence was detected by exciting the samples using the blue LED set at 85% excitation power in each microfluidic well. The fluorescence and OD<sub>600</sub> values were recorded every 30 min.

**Closed-Loop Experiments.** Protocols to prepare samples, to load samples into the well plate or microfluidic device, and to set up the optogenetics system followed the procedures of the open-loop experiment. An in-house Python script was written to implement the PID control algorithm similar to our previous work.<sup>51</sup> The PID controller contains three gain coefficients:  $K_{\rm P}$ ,  $K_{\rm L}$ , and  $K_{\rm D}$  for the proportional, integral, and derivative terms in the PID controller equation. For closedloop experiments, the measurements and controller's output were calculated every 30 min. The gains for each experiment were set to the following:  $K_{\rm P} = 6$  or 7 and  $K_{\rm I} = 0.5$  or 0.2, which were chosen by the root locus plots (Figure S10).  $K_{\rm D}$ was set to 0 since the derivative term represents anticipatory control, which was not required for our experiments. Table S5 describes all of the parameters used for each closed-loop experiment. Mathematically, the closed-loop control followed a five-step algorithm presented in Figure S9.

**Statistics.** Tolerance bands ( $\pm 15\%$ ) were added to the setpoint of the closed-loop experiments to show reproducibility and to show the correlation of the output to the setpoint



**Figure 1.** CcaSR sensitivity to blue light. (A) Fluorescence accumulation of GFP over 24 h due to periodic activation (blue) and deactivation (red) of the CcaSR system every 5 h. (B) Illumination profile: induction (shaded blue) and repression (shaded red) cycles and the rate of change in the GFP expression rate (shaded green). The shaded green area represents the average and  $\pm 1$  S.D with n = 4. (C) Graph showing changes in OD during the experiment. (D) Normalized amount of accumulated GFP over OD represented as a bar plot. A fluorescence and OD<sub>600</sub> value were obtained at the end of every red or blue light irradiation cycle. A Student's *t*-test (p = 0.02 < 0.05) was performed on the normalized fluorescence values over the red light cycles to show the effect of blue light.

for graphs in Figures 3A,D, 4D, and S14 and S15. In experiments with multiple setpoints (Figure 3A), a paired, twotailed, t-test was performed by selecting an equal set of values (n = 10) within the tolerance margins for each setpoint (i.e., 8 and 12 a.u/h). A normalized cross-correlation coefficient (time-dependent Pearson correlation coefficient) was determined between the setpoint and the response. The steady-state error was calculated for each closed-loop experiment (Table S5) by calculating the absolute value of the average error between the setpoint and the response. The steady-state value was measured for each experiment after oscillations in the response converged on  $\pm 15\%$  of the setpoint. All statistical calculations were performed on Excel using functions T-TEST (for t-test) and CORREL (for Pearson correlation coefficient).

## RESULTS AND DISCUSSION

Characterization of the RT-OGENE System. In the "real-time optogenetics" (RT-OGENE) system (for design and setup, see the Supporting Information; Figures S1-S4), we used green and red light ( $\lambda$  = 520 and 650 nm) to dose the CcaSR system, respectively, and blue light ( $\lambda = 480$  nm) to excite GFP (the reporter expressed by the CcaSR system). While the optimal induction spectrum of the CcaSR overlaps with green light, the spectrum also overlaps (to a lesser extent) with blue light ( $\lambda$  = 480 nm) used for GFP excitation (Figure S5A,B). We hypothesized that the activation and emission of the CcaSR system under blue light excitation (instead of the green light) will also induce protein expression. As an initial test, we have alternated exposure of samples to blue and red light periodically every 5 h, while measuring fluorescence every hour. As shown in Figure 1A, GFP levels clearly increase while samples are under blue light. Similarly, the GFP expression rate increases during blue light exposure (a 2-4-fold increase compared to red light exposure) (Figure 1B). We also normalized the fluorescence measurement by the OD<sub>600</sub> measurement (Figure 1C) to determine if the increase in fluorescence is due to growth, and as expected, blue light is the cause for the increases in fluorescence over time (P < 0.05; Figure 1D). Although blue light induces GFP expression, it is

only after 5 h this is observed. Hence, to minimize the effect of the blue light on the CcaSR system, we only activated the blue light for a short duration (650 ms) to measure the GFP fluorescence and continued to use green and red lights to regulate the CcaSR system.

A key feature of the RT-OGENE system is the ability to control multiple samples independently through simultaneous activation of multiple lights. Controlling the gene expression of multiple samples require the simultaneous activation of several LEDs. LEDs are a popular light source for optogenetics given they are cheap, and their small footprint leads to easy integration with other equipment (e.g., microscope). The problem with LEDs is that the light spreads in every direction, which can generate light crosstalk adjacent to the target sample (Figure S6A). This problem is even magnified if we are trying to illuminate samples that are close in proximity (especially in microfluidic devices<sup>42</sup>). This problem can be solved using the methods described here; on our system, the light from the LED is guided through a beam narrow plate (containing small apertures) that will block light into the adjacent wells (Figure S6B). As shown in Figure S6C, we measured the light intensity in an adjacent well for the green and red light with a beam narrow plate and obtained a very minimal crosstalk 0.008 and 0.08%, respectively (compared to 0.05 and 0.5% without the beam narrow plate). We further tested light crosstalk with cell samples transformed with the CcaSR system, and as expected, the measured fluorescence intensities (after 6 h of constant green illumination) are minimal in the adjacent well (Figure S6D). Although we observe a fold-change in the fluorescence in the adjacent (and dark) wells, we hypothesize that this is due to the leaky transcription of the system<sup>25</sup> together with cell growth during the 6 h and not due to light crosstalk (see Figures S7 and S8 for OD/fluorescence calibration of our system in the Supporting Information). Considering this result, we propose that future work will incorporate an optimized variant of the CcaSR system with reduced leaky expression<sup>27</sup> and improved light delivery such as direct integration of fibers or collimating optics to reduce the light bleed in adjacent wells.<sup>52</sup>

#### pubs.acs.org/ac



**Figure 2.** Tuning RT-OGENE via open-loop experiments. The fluorescence and absorbance profiles of transformed CcaSR bacteria grown under different green and red light illumination profiles. (A) Constant: experimental and simulated profiles of fluorescence when bacteria were grown under three conditions: 0% green light, 30% green light (i.e., cycles 3 min green and 7 min red), and 45% green light (i.e., cycles of 4.5 min green and 5.5 min red) for the full duration of the experiment of 7 h. The model was manually tuned to fit the experiment results. (B) OD<sub>600</sub> profile for constant inducer experiment (n = 3). (C) Pulsed: experimental (green solid) and simulated (black solid) profiles of fluorescence when bacteria were grown under green and red light at 40% duty cycle with a period time of 10 min for 4 h. The activation pulse is indicated by a dotted black line. (D) OD<sub>600</sub> profile for the pulsed experiment (n = 4). (E) Random: experimental (green solid) and simulated (black solid) profiles of fluorescence when bacteria were grown under various green and red light between 0 and 90% duty cycle with a period time of 10 min for the full duration of the experiment of 16 h. The activation pulse is indicated by a dotted black line. (F) OD<sub>600</sub> profile for the pulsed experiment (n = 4). For all absorbance curves, the solid line represents the average of the absorbance measurements and the red shaded region indicates  $\pm$  one standard deviation of the average.

Model-Based Design. After optimizing our RT-OGENE system, we turned our attention to implementing the modelbased design (MBD) methodology toward the CcaSR system. Although there are optogenetic systems for analyzing gene expression (in plates/tubes<sup>38</sup> and in microfluidics<sup>53</sup>), we are not aware of any publications using an MBD approach in this capacity. MBD is well suited for this task because it enables developers to test and verify their solution at early stages of design via modeling rather than endure a prolonged trial-anderror process using a complex biological system (like CcaSR).<sup>54</sup> For example, we can model the "plant" (the CcaSR) system via differential equations (Tables S1 and S2) and rapidly find a tuned controller suitable for controlling gene expression instead of trial-and-error experimentation to tune the system toward the desired output.<sup>41,55</sup> Our approach can be a cheaper and more efficient approach to implementing feedback control of gene expression using optogenetics (for the detailed MBD methodology, see Figure S9).

To obtain initial values for our plant, we followed Ang et al.<sup>56</sup> to find the transcription, translation, and decay coefficients to match the rise and fall times of GFP expression. We executed an open-loop experiment under a mixture of green/red light (0/100, 30/70, 45/55%) for 7 h to find initial values of our plant such that the model is tuned to fit the experimental results (Tables S3 and S4). Using this method, we are able to achieve a model that shows similar responses to the experiment (Figure 2A). We also measured the OD (Figure 2B) to determine if the low rate of expression at 100% red light is due to growth or leakiness of the promoter.<sup>27</sup> We compared the normalized GFP/OD values at 1, 3, and 5 h and obtained different values (16.5, 18.5, and 20, respectively), which suggests that the increase is most likely due to the leakiness of the CcaSR system, which we included in our plant

model. We also validated the robustness of our plant by executing other illumination schemes (pulsed and random). Generally, we observe similar trends in the fluorescence output for the plant compared to the pulsed (Figure 2C,D) and random (Figure 2E,F) experiments. However, given the day-to-day variability of the cells and the global metabolic changes to the cells, this can cause a variety of unexpected expression trajectories.<sup>41</sup> For example, cells will exhibit different rates of (de)phosphorylation, which can contribute to the differences between our plant and experiment and may improve by refining the plant in future work. Regardless, the similar trends between the model and experiment establish a plant that can be used to tune our controller *in silico*.

The controller that we used for this work is a PID. We used a root locus method to determine gain coefficients ( $K_{\rm P} = 6.0$ ,  $K_{\rm I}$  = 0.5, and  $K_{\rm D}$  = 0) for the controller, which is connected to the plant in our model. Through simulations, we verified that the controller is stable (i.e., poles are within the unit circle; Figure S10A) and it maintains a high gain margin ( $\sim$ 6.8) to account for the variability in the cell responses. To validate the controller's performance, we simulated a discrete closed-loop system to track a constant reference expression rate (Figure S11A). As shown in Figure S11B, three different expression rates were simulated: 4, 8, and 16 a.u/h and all rates reached their target value after ~11 h (measured within 2% of the target). The chosen K values (a  $K_p/K_i$  ratio of ~12) show fast convergence with minimal overshoot (~15%) for all three tested expression rates of 4, 8, and 16 a.u/h and this result is comparable to other PID controllers used to control the CcaSR system.<sup>41</sup> Hence, we show that we can model the CcaSR, tune the controller without trial-and-error experimentation, and use it for the closed-loop experiments described below.



**Figure 3.** Closed-loop step response. Comparison of fluorescence and absorbance profiles of transformed CcaSR bacteria grown under closed-loop control for a (A–C) low target setpoint and a (D–F) high target setpoint. (A) Expression rate setpoint of GFP was set to 8 a.u./h for 14 h and then increased to 12 a.u./h (red line; n = 4) with tolerance margins  $\pm 15\%$  (dotted red lines). The real-time tracking of the GFP expression rate is indicated by a black line. Statistical analysis shows a significant change in the output when the setpoint is changed ( $p \ll 0.01$ ). A maximum normalized cross-correlation coefficient of 0.825 was determined between the setpoint and response signals at an offset of 2 h. (B) Overall accumulation of GFP in the system (green curve) and the illumination profile (shaded green and red areas) for the low rate setpoint. (C) OD<sub>600</sub> profile for the bacteria cells controlled at the low rate setpoint. (D) Expression rate is indicated by a black line. (E) Overall accumulation of GFP in the system (green curve) and the illumination profile (shaded green and red areas) for the high rate setpoint. (F) OD<sub>600</sub> profile for the bacteria cells controlled at the low rate setpoint. For all absorbance curves, the solid line represents the average of the absorbance measurements and the red shaded region indicates  $\pm$  one standard deviation of the average. Error bars also represent  $\pm 1$  S.D with n = 4.

**Closed-Loop Experiments.** In microbes such as *E.coli*, the expression of enzymes must be finely tuned and timed to maximize the yield of valuable products.<sup>57–59</sup> With this motivation (and to verify our MBD approach), we integrated the tuned PID controller with our RT-OGENE system to control GFP expression.<sup>56</sup> In the first test, the expression level of GFP was set to a low expression rate (8 a.u/h) for 14 h and the system monitored the rate of fluorescence and the illumination profiles (see Figure S12 for the algorithm). As shown in Figure 3A, the rate immediately starts to increase toward the setpoint by activating the inducer (i.e., green light). At 3 h, there is a high overshoot from the target by  $\sim 90\%$ ; however, the system activates the repressor (i.e., red light) to reduce the rate back to the target when the overshoot starts to occur and the rate starts to converge around the setpoint  $(\pm 30\%)$  after 6 h (Figure 3B). We further increased the setpoint to 12 a.u/h at 14 h and the system was able to respond toward the target. A t-test analysis of the GFP response data under these different setpoints shows that they are significant ( $p \ll 0.01$ ). A normalized cross-correlation coefficient of 0.823 between the setpoint signal and the GFP response shows excellent correlation, but cells are slow to respond to the light activation due to the time delay of 2 h (i.e., the delay of the GFP expression rate to the changing setpoint) to obtain this correlation. During the 24 h, we observe a total fluorescence accumulation and a growth rate ( $\sim 0.07 \text{ h}^{-1}$ ) similar to the previous open-loop control experiments (Figure 3C). Next, we set the target rate to a higher expression rate of 22 a.u/h. As expected, the GFP accumulated rapidly toward the setpoint and reached the target after 2 h (Figure 3D).

Similar to the lower setpoint, once the target is reached, there is a high overshoot (~90%), but the red light is activated to reduce the overshoot (Figure 3E). Typically, PID controllers require very high gain settings to achieve such a fast rise, which inevitably will also result in large oscillations and overshoot.<sup>41</sup> We explored solutions for reducing the overshoot, namely, by tuning the PID parameters (Figure S13A) and changing the setpoint gradually (Figure S13B). Additionally, different controllers may provide a decreased overshoot when applied to our hardware.<sup>42,60</sup> Despite the overshoot, we do achieve the target expression rate (Figure 3A,D) and stable growth of the cells (Figure 3C,F).

Having established a closed-loop controller with the RT-OGENE system to simultaneously regulate expression for samples with the same setpoint, we applied the RT-OGENE system to regulate expression at two different setpoints to further investigate our system's performance. Generally, there are only reports of controlling one setpoint<sup>41</sup> and as far as we are aware, this is the first report of an optogenetic method that is designed to control two different setpoints simultaneously. We have prepared four bacterial cultures at a starting OD of 0.3 a.u. and two wells were set to a constant rate of 14 a.u/h and two different wells were set to a constant rate of 28 a.u/h. As shown in Figure S14A,B, the system steadily increased toward their setpoints with an overshoot of 30 and 80%. As expected, the higher setpoint showed a higher overshoot and required a longer duration to stabilize near the target. The stabilization is more prominent from the light input-as more oscillations (between the inducer and repressor) are shown when the setpoint is reached (Figure S14C,D). The GFP

Article



**Figure 4.** Microfluidic platform. Testing the RT-OGENE system with a microfluidic platform. (A) Image of the microfluidic device used for openand closed-loop experiments. Open-loop response. (B) GFP accumulation in response to different inducer strengths (10, 20, 40, and 60%) in the four microfluidic wells. 10% represents cycles of 1 min green and 9 min red. (C) Averaged OD<sub>600</sub> profile for the bacteria cells in response to 10, 20, and 60% inducer. Closed-loop response. (D) Output GFP expression rate (solid black line) at a target setpoint of 8 a.u./h (solid red line) with tolerance margins  $\pm$  15% (dotted red lines). (E) Overall accumulation of GFP in the system (green curve) and the illumination profile (shaded green and red areas) for the closed-loop control experiment. Error bars also represent  $\pm$  1 S.D. with n = 4.

accumulation also shows increases in both cases with the higher target exhibiting higher fluorescence values. We extended our experiments to track and to control four different setpoints (0, 4, 6, and 9 a.u/h) and the results are consistent with the above set of closed-loop experiments (Figure S14E,F). Finally, we inoculated the starting culture at different  $OD_{600}$ values (0.35 and 0.55) to determine if the starting density will affect the tracking behavior. When samples contained more bacteria at the start (Figure S15A), it showed a steeper increase in the GFP expression rate during the first 5 h and it also reached its setpoint faster (9 vs 10 h). We also observed a smaller overshoot (120%) in contrast to samples with lower starting OD (250%; Figure S15B). These trends are expected-since cell density (which is expressing GFP) can reach the target values faster if the cells are outputting a fluorescence close to the target setpoint.<sup>61</sup> Although the model gene used here (GFP) is well characterized, we propose in the future that the MBD approach may be used to rapidly design the closed-loop optogenetics system to control other genes, reducing the time and costs associated with trial-and-error experimentation.

Microfluidic Platform. Microfluidics is becoming popular to analyze single-cell responses using optogenetics.<sup>4</sup> Here, we want to show the versatility of our system, i.e., the RT-OGENE is well suited for well plates and adaptable to other platforms like microfluidics. We fabricated a device (Figure 4A) that consists of one continuous channel, shaped in a Uconfiguration with four incubation chambers that are 1.5 mm in height (translates to ~ 2.25  $\mu$ L of culture media). We applied our RT-OGENE system to monitor the rate of gene expression of GFP and cell growth in the microfluidic chambers. As expected, fluorescence measurements at 60% inducer strength produced the highest production rate after 20 h and at 10% the fluorescence values showed the lowest production rate (Figure 4B). In addition, we have measured the OD for each well and have observed high variations in the average OD (Figure 4C). We speculate that the variations in the OD measurements may be caused by several factors (path length, optics, etc). The most likely is that the microfluidic channel is connected and there is no separation between the

wells, which will allow bacteria to diffuse to other wells in the channel, which can vastly affect the OD readings in each well.

We also integrate the microfluidics to control gene expression in the microfluidic wells using the same previously tuned controller. We initially exposed the samples to green light and set a target rate of 6 a.u/h. Although the volumes of the samples were 40 times smaller, they all reached the target setpoint after  $\sim 8$  h, which is similar to the trends seen in the well-plate experiments (Figure 4D). The similarities in the overshoot and steady-state error between the microfluidics and well plates indicate that our system can be used to track expression levels in samples cultured in microfluidic devices. Furthermore, because our platform processes fluorescence data real time, it can monitor the illumination inputs as well as informing the total accumulation of GFP in the system (Figure 4E). In future work, we plan to make improvements to the device itself to reduce both leakage and bacterial diffusion using a mother machine microfluidic device that is capable of examining optogenetic circuits in a single-cell format<sup>62,63</sup> or the use of digital microfluidics given their inherent ability for individual addressability and control.<sup>64</sup> Using these platforms, the variability in the measurements (especially in OD) could be decreased while still allowing many samples to be controlled via our optogenetics system.

## CONCLUSIONS

A real-time optogenetics platform for tracking gene expression under open- and closed-loop control has been developed using a standard camera, 3D-printed parts, and an electronic board to house a matrix of LEDs and switches. This optogenetics system operates using light to control gene expression levels in four bacterial samples through measurement of the optical density and fluorescence of the cells in culture. We are the first to use a model-based design approach to model the CcaSR system and tune our controller *in silico* without trial-and-error experimentation. We verified the approach by applying it to controlling the gene expression levels of GFP. We believe that this new approach may be beneficial for other laboratories interested in using optogenetics to control gene expression of different genes and many samples and conditions.

## **Analytical Chemistry**

#### ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c04594.

Reagents and plasmids; light delivery system, operation and calibration; bacterial cryopreservation, preparation, culture, and transformation; microfluidic device fabrication; comprehensive model of the CcaSR system; The nonlinear, time-variant, single-in model; linear and timeinvariant, single-in single-out model (Methodology); equations for the comprehensive, nonlinear, time-variant model (Table S1); equations for the nonlinear, timevariant, single-in model (Table S2); values used for the nonlinear, time-variant, single-in model (Table S3); state-space representation of the model (Table S4); parameters and conditions used for closed-loop experiments (Table S5); equations for power compensation (Table S6); TECAN configuration for OD measurements (Table S7); TECAN configuration for fluorescence measurements (Table S8); optogenetic optical design (Figure S1); RT-OGENE automation system connectivity (Figure S2); light delivery connectivity (Figure S3); real-time monitoring of the optogenetics experiment (Figure S4); light delivery spectrum (Figure S5); beam narrower setup and characterization (Figure S6); OD<sub>600</sub> calibration using RT-OGENE (Figure S7); fluorescence calibration results (Figure S8); sequence of steps to implement the model-based design methodology (Figure S9); root locus (Figure S10); modeling in discrete time domain (Figure S11); digital signal processing algorithm (Figure S12); reducing overshoot (Figure S13); simultaneous control of samples with different setpoints (Figure S14); regulating the protein expression rate when starting at different OD<sub>600</sub> values (Figure S15) (PDF)

Software showing real-time fluorescence and OD measurements using RT-OGENE (Movie S1) (MP4)

## AUTHOR INFORMATION

#### **Corresponding Author**

Steve C. C. Shih – Department of Electrical and Computer Engineering, Centre for Applied Synthetic Biology, and Department of Biology, Concordia University, Montréal, Québec H3G1M8, Canada; orcid.org/0000-0003-3540-0808; Phone: (514) 848-2424; Email: steve.shih@ concordia.ca

#### Authors

- **Guy Soffer** Department of Electrical and Computer Engineering and Centre for Applied Synthetic Biology, Concordia University, Montréal, Québec H3G1M8, Canada
- James M. Perry Centre for Applied Synthetic Biology and Department of Biology, Concordia University, Montréal, Québec H4B1R6, Canada

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c04594

#### Author Contributions

G.S. and J.M.P. contributed equally to this work. The research was designed by G.S., J.M.P., and S.C.C.S. All experiments and analyses were conducted by G.S., J.M.P., and S.C.C.S. Bacterial culture work (cell passaging, maintenance) and biological

methods were performed by J.M.P. The mathematical modeling, simulations, and controller design were done by G.S. The mechanical, electrical, and software designs were developed by G.S. All authors discussed the results, wrote, and reviewed the manuscript.

#### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council (NSERC), the Fonds de Recherche Nature et Technologies (FRQNT), and the Canadian Foundation of Innovation (CFI) for funding. G.S. thanks the Concordia University Department of Electrical and Computer Engineering for FRS Funding and the department of Biology for academic resources. SCCS thanks Concordia for a University Research Chair.

### REFERENCES

(1) Teh, M. Y.; Ooi, K. H.; Danny Teo, S. X.; Bin Mansoor, M. E.; Shaun Lim, W. Z.; Tan, M. H. ACS Synth. Biol. **2019**, *8*, 708–723.

- (2) Bourgeois, L.; Pyne, M. E.; Martin, V. J. J. ACS Synth. Biol. 2018, 7, 2675–2685.
- (3) Lee, M. E.; DeLoache, W. C.; Cervantes, B.; Dueber, J. E. ACS Synth. Biol. 2015, 4, 975–986.

(4) Mutalik, V. K.; Guimaraes, J. C.; Cambray, G.; Lam, C.; Christoffersen, M. J.; Mai, Q. A.; Tran, A. B.; Paull, M.; Keasling, J. D.; Arkin, A. P.; Endy, D. Nat. Methods **2013**, 10, 354–360.

(5) Markley, A. L.; Begemann, M. B.; Clarke, R. E.; Gordon, G. C.; Pfleger, B. F. ACS Synth. Biol. 2015, 4, 595–603.

(6) An-Adirekkun, J. M.; Stewart, C. J.; Geller, S. H.; Patel, M. T.; Melendez, J.; Oakes, B. L.; Noyes, M. B.; McClean, M. N. *Biotechnol. Bioeng.* **2020**, *117*, 886–893.

(7) Zha, W.; An, T.; Li, T.; Zhu, J.; Gao, K.; Sun, Z.; Xu, W.; Lin, P.; Zi, J. ACS Synth. Biol. **2020**, *9*, 449–456.

(8) Klingl, S.; Kordes, S.; Schmid, B.; Gerlach, R. G.; Hensel, M.; Muller, Y. A. Protein Expression Purif. 2020, 172, No. 105632.

(9) Toettcher, J. E.; Gong, D.; Lim, W. A.; Weiner, O. D. Nat. Methods 2011, 8, 837-839.

(10) Bittihn, P.; Didovyk, A.; Tsimring, L. S.; Hasty, J. Nat. Microbiol 2020, 5, 697–705.

(11) Corrales-Garcia, L. L.; Serrano-Carreon, L.; Corzo, G. Protein Expression Purif. **2020**, 167, No. 105539.

(12) Ryo, S.; Ishii, J.; Matsuno, T.; Nakamura, Y.; Matsubara, D.; Tominaga, M.; Kondo, A. ACS Synth. Biol. 2017, 6, 928–935.

(13) Gomes, L.; Monteiro, G.; Mergulhao, F. Int. J. Mol. Sci. 2020, 21, No. 576.

(14) Dvorak, P.; Chrast, L.; Nikel, P. I.; Fedr, R.; Soucek, K.; Sedlackova, M.; Chaloupkova, R.; de Lorenzo, V.; Prokop, Z.; Damborsky, J. *Microb. Cell Fact.* **2015**, *14*, No. 201.

(15) Kelly, C. L.; Liu, Z.; Yoshihara, A.; Jenkinson, S. F.; Wormald, M. R.; Otero, J.; Estevez, A.; Kato, A.; Marqvorsen, M. H.; Fleet, G. W.; Estevez, R. J.; Izumori, K.; Heap, J. T. ACS Synth. Biol. 2016, 5, 1136–1145.

(16) Chappell, J.; Takahashi, M. K.; Lucks, J. B. Nat. Chem. Biol. 2015, 11, 214–220.

(17) Green, A. A.; Silver, P. A.; Collins, J. J.; Yin, P. Cell 2014, 159, 925–939.

(18) Thakore, P. I.; D'Ippolito, A. M.; Song, L.; Safi, A.; Shivakumar, N. K.; Kabadi, A. M.; Reddy, T. E.; Crawford, G. E.; Gersbach, C. A. *Nat. Methods* **2015**, *12*, 1143–1149.

(19) Chou, L. Y. T.; Shih, W. M. ACS Synth. Biol. 2019, 8, 2558-2565.

(20) Westbrook, A. M.; Lucks, J. B. Nucleic Acids Res. 2017, 45, 5614–5624.

(21) Stevens, J. T.; Carothers, J. M. ACS Synth. Biol. 2015, 4, 107–115.

(22) Gordon, G. C.; Korosh, T. C.; Cameron, J. C.; Markley, A. L.; Begemann, M. B.; Pfleger, B. F. *Metab Eng* **2016**, *38*, 170–179.

- (23) Re, A. Front. Cell Dev. Biol. 2017, 5, No. 77.
- (24) Jayaraman, P.; Devarajan, K.; Chua, T. K.; Zhang, H.; Gunawan, E.; Poh, C. L. Nucleic Acids Res. 2016, 44, 6994-7005.
- (25) Schmidl, S. R.; Sheth, R. U.; Wu, A.; Tabor, J. J. ACS Synth. Biol. 2014, 3, 820-831.
- (26) Ong, N. T.; Olson, E. J.; Tabor, J. J. ACS Synth. Biol. 2018, 7, 240-248.
- (27) Ong, N. T.; Tabor, J. J. ChemBioChem 2018, 19, 1255-1258.
- (28) Meador, K.; Wysoczynski, C. L.; Norris, A. J.; Aoto, J.; Bruchas, M. R.; Tucker, C. L. Nucleic Acids Res. 2019, 47, No. e97.
- (29) Uda, Y.; Goto, Y.; Oda, S.; Kohchi, T.; Matsuda, M.; Aoki, K. Proc. Natl. Acad. Sci. U.S.A. **2017**, 114, 11962–11967.
- (30) Salinas, F.; Rojas, V.; Delgado, V.; Lopez, J.; Agosin, E.; Larrondo, L. F. *mBio* **2018**, *9*, No. e00626-18.
- (31) Gerhardt, K. P.; Olson, E. J.; Castillo-Hair, S. M.; Hartsough, L.
- A.; Landry, B. P.; Ekness, F.; Yokoo, R.; Gomez, E. J.; Ramakrishnan, P.; Suh, I.; Savage, D. F.; Tabor, I. J. *Sci Rep* **2016**, *6*, No. 35363.
- (32) Liu, Z.; Zhang, J.; Jin, J.; Geng, Z.; Qi, Q.; Liang, Q. Front. Microbiol. 2018, 9, No. 2692.
- (33) Mansouri, M.; Strittmatter, T.; Fussenegger, M. Adv. Sci. 2019, 6, No. 1800952.
- (34) Zhao, E. M.; Zhang, Y.; Mehl, J.; Park, H.; Lalwani, M. A.; Toettcher, J. E.; Avalos, J. L. *Nature* **2018**, 555, 683–687.
- (35) Fenno, L.; Yizhar, O.; Deisseroth, K. Annu. Rev. Neurosci. 2011, 34, 389–412.
- (36) Sheets, M. B.; Wong, W. W.; Dunlop, M. J. ACS Synth. Biol. 2020, 9, 227–235.
- (37) Wang, H.; Yang, Y. T. ACS Synth. Biol. 2017, 6, 1793-1796.
- (38) Davidson, E. A.; Basu, A. S.; Bayer, T. S. J. Mol. Biol. 2013, 425, 4161-4166.
- (39) Fracassi, C.; Postiglione, L.; Fiore, G.; di Bernardo, D. ACS Synth. Biol. 2016, 5, 296–302.
- (40) Rullan, M.; Benzinger, D.; Schmidt, G. W.; Milias-Argeitis, A.; Khammash, M. *Mol. Cell* **2018**, *70*, 745–756.
- (41) Milias-Argeitis, A.; Rullan, M.; Aoki, S. K.; Buchmann, P.; Khammash, M. Nat. Commun. **2016**, 7, No. 12546.
- (42) Chait, R.; Ruess, J.; Bergmiller, T.; Tkacik, G.; Guet, C. C. Nat. Commun. 2017, 8, No. 1535.
- (43) Melendez, J.; Patel, M.; Oakes, B. L.; Xu, P.; Morton, P.; McClean, M. N. Integr. Biol. 2014, 6, 366-372.
- (44) Menolascina, F.; Fiore, G.; Orabona, E.; De Stefano, L.; Ferry, M.; Hasty, J.; di Bernardo, M.; di Bernardo, D. *PLoS Comput. Biol.* **2014**, *10*, No. e1003625.
- (45) Olson, E. J.; Hartsough, L. A.; Landry, B. P.; Shroff, R.; Tabor, J. J. Nat. Methods **2014**, *11*, 449–455.
- (46) Ruess, J.; Parise, F.; Milias-Argeitis, A.; Khammash, M.; Lygeros, J. Proc. Natl. Acad. Sci. U.S.A. **2015**, *112*, 8148–8153.
- (47) Uhlendorf, J.; Miermont, A.; Delaveau, T.; Charvin, G.; Fages, F.; Bottani, S.; Batt, G.; Hersen, P. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, 109, 14271–14276.
- (48) Bugaj, L. J.; Lim, W. A. Nat Protoc 2019, 14, 2205-2228.
- (49) Reedy, J.; Lunzman, S. Model Based Design Accelerates the
- Development of Mechanical Locomotive Controls, 0148-7191, SAE Technical Paper; SAE, 2010.
- (50) Hirose, Y.; Shimada, T.; Narikawa, R.; Katayama, M.; Ikeuchi, M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9528–9533.
- (51) Moazami, E.; Perry, J. M.; Soffer, G.; Husser, M. C.; Shih, S. C. C. Anal. Chem. **2019**, *91*, 5159–5168.
- (52) Yang, H.; Gijs, M. A. M. Chem. Soc. Rev. 2018, 47, 1391–1458. (53) Chait, R.; Ruess, J.; Bergmiller, T.; Tkačik, G.; Guet, C. C. Nat. Commun. 2017, 8, No. 1535.
- (54) Crook, N.; Alper, H. S. Chem. Eng. Sci. 2013, 103, 2-11.
- (55) Milias-Argeitis, A.; Summers, S.; Stewart-Ornstein, J.; Zuleta, I.; Pincus, D.; El-Samad, H.; Khammash, M.; Lygeros, J. *Nat. Biotechnol.* **2011**, *29*, 1114–1116.
- (56) Ang, J.; Harris, E.; Hussey, B. J.; Kil, R.; McMillen, D. R. ACS Synth. Biol. 2013, 2, 547–567.

- (57) Ajikumar, P. K.; Xiao, W. H.; Tyo, K. E.; Wang, Y.; Simeon, F.; Leonard, E.; Mucha, O.; Phon, T. H.; Pfeifer, B.; Stephanopoulos, G. *Science* **2010**, *330*, 70–74.
- (58) Nicolaou, S. A.; Gaida, S. M.; Papoutsakis, E. T. Metab. Eng. 2010, 12, 307-331.
- (59) Peabody, G. L.; Winkler, J.; Kao, K. C. Curr. Opin. Chem. Eng. 2014, 6, 9–17.
- (60) Fiore, G.; Perrino, G.; di Bernardo, M.; di Bernardo, D. ACS Synth. Biol. 2016, 5, 154–162.
- (61) Kawasaki, S.; Borchert, C.; Deyholos, M.; Wang, H.; Brazille, S.; Kawai, K.; Galbraith, D.; Bohnert, H. J. *Plant Cell* **2001**, *13*, 889–905.
- (62) Lugagne, J. B.; Dunlop, M. J. Curr. Opin. Syst. Biol. 2019, 14, 1-8.
- (63) Potvin-Trottier, L.; Luro, S.; Paulsson, J. Curr. Opin. Microbiol. 2018, 43, 186-192.
- (64) Husser, M. C.; Vo, P. Q. N.; Sinha, H.; Ahmadi, F.; Shih, S. C. C. ACS Synth. Biol. 2018, 7, 933–944.