Design and Kinetic Analysis of a Hybrid Promoter−Regulator System for Malonyl-CoA Sensing in Escherichia coli

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Supporting Information

ABSTRACT: Malonyl-CoA is the rate-limiting precursor involved in the chain elongation reaction of a range of value-added pharmaceuticals and biofuels. Development of malonyl-CoA responsive sensors holds great promise in overcoming critical pathway limitations and optimizing production titers and yields. By incorporating the Bacillus subtilis trans-regulatory protein FapR and the cis-regulatory element fapO, we constructed a hybrid promoter−regulatory system that responds to a broad range of intracellular malonyl-CoA concentrations (from 0.1 to 1.1 nmol/mgDW) in Escherichia coli. Elimination of regulatory protein and nonspecific DNA cross-communication leads to a sensor construct that exhibits malonyl-CoA-dependent linear phase kinetics with increased dynamic response range. The sensors reported in this study could potentially control and optimize carbon flux leading to robust biosynthetic pathways for the production of malonyl-CoA-derived compounds.

Recent efforts in synthetic biology have focused on the development of genetically encoded components that support the design, construction, and optimization of biological systems.1,2 However, the precise control and regulation of heterologous pathway expression when environmental or intracellular conditions change remains an engineering conundrum. Despite the fact that inducible expression systems (i.e., IPTG/T7 or arabinose/pBAD) have been widely used for controlling gene expression in metabolic pathway engineering, their rigid control over the whole system makes them inefficient to use in order to adjust the host metabolic function based on the changing environment.3 Therefore, engineering of sensor−regulator systems that can respond to a particular precursor metabolite and actuate the desired cellular response would allow the cell to efficiently utilize cellular resources and improve production titers and yields.4 For example, Farmer and Liao have designed and engineered a regulatory circuit that could sense the glycolytic pathway hallmark metabolite acetyl-CoA and actuate the desired cellular response.5 Recently, a fatty acyl-CoA responsive promoter has been developed to dynamically modulate gene expression involved in biodiesel pathways; the resulting dynamic sensor−regulator system has led to a 3-fold increase in fatty acid ethyl ester (FAEE) production compared with using constitutive promoters in Escherichia coli.6 Nature possesses tremendous potential for the production of pharmaceutical compounds and biofuels through the plethora of chemistries that exist in different organisms.7−9 One particular synthetic chemistry endowed by nature is the decarboxylative carbon condensation reaction using malonyl coenzyme A (malonyl-CoA) as carbon donor.10 For example, malonyl-CoA is the basic building block for the biosynthesis of an array of value-added compounds including fatty acids,11 phenylpropanoids,12,13 and polyketides.14 Cell-free biochemical studies have demonstrated that malonyl-CoA is the major rate-limiting precursor for synthesizing fatty acids.15,16 In the past decade, substantial effort has been made to engineer the central metabolic pathways of E. coli in order to improve the intracellular level of malonyl-CoA.17−19 As such, developing an engineered malonyl-CoA responsive biosensor offers new opportunities to overcome critical pathway limitations for the efficient biosynthesis of pharmaceutical intermediates and biofuels.

Based on the findings of Schujman and co-workers,20,21 FapR is a naturally existing transcriptional regulator involved in the fatty acid biosynthetic pathway of the Gram-positive bacterium Bacillus subtilis. Native FapR has two functional domains: the C-terminal ligand-binding domain that specifically recognizes and binds malonyl-CoA and the N-terminal regulatory domain that binds to a short DNA fragment, fapO (operator region on...
the *fap* regulon), and modulates the expression of downstream genes. Binding of FapR to *fapO* will block the access of RNA polymerase and thus repress transcription of the downstream fatty acid pathway. On the other hand, binding of malonyl-CoA to the C-terminal domain of FapR would promote a conformational change in FapR that would propagate to the N-terminal domain and lead to its dissociation from *fapO*, thus relieving this transcriptional repression. Recently, a FapR-based malonyl-CoA sensor has been developed to detect changes of malonyl-CoA flux in living mammalian cells.22 However, in *E. coli*, there is no reported regulatory protein that can specifically recognize and respond to malonyl-CoA.

Here we report engineering a hybrid promoter—regulator system that could respond to a broad range of intracellular malonyl-CoA (from 0.1 to 1.1 nmol/mgDW). Dynamic transcriptional activity and surface plasmon resonance analysis revealed that FapR cross-interacts with the commonly used lactose repressor binding site *lacO*. Elimination of the cross-talking *lacO* site could convert the parabolic biphasic response kinetics to single-phase linear kinetics. The constructed malonyl-CoA sensor could be used to dynamically regulate metabolic pathways that are involved in the biosynthesis of important compounds.

### RESULTS AND DISCUSSION

**Design of Sensor That Responds to Both Malonyl-CoA and IPTG.** We first opted to integrate the *B. subtilis* fapR—malonyl-CoA regulation mechanism into an *E. coli* T7 promoter system and test whether the constructed promoter—regulator would respond to malonyl-CoA. A schematic representation of the hybrid promoter—regulator system is shown in Figure 1. In the constructed genetic circuits, codon optimized *fapR* was expressed under the control of a T7 promoter. The FapR binding site, the operator *fapO* (∼35 bp), was inserted downstream of the T7 promoter and lacO operator. Enhanced green fluorescence protein (eGFP) was used as a reporter and expressed under the control of this hybrid T7 promoter (T7 promoter with *fapO*). The expression of eGFP was expected to be regulated by both IPTG and malonyl-CoA inside the cell. For example, when the malonyl-CoA level is low, FapR was expected to bind to *fapO*, block the access of T7 RNA polymerase and repress the expression of eGFP. Conversely, when the malonyl-CoA level is high, binding of malonyl-CoA with FapR should disrupt the interaction between FapR and *fapO*, and therefore the transcriptional repression should be relieved. In addition to the above-mentioned sensor, we also attempted to test whether the introduced *fapO* or *fapR* would interfere with the transcriptional activity of the original T7 promoter. For this reason, we constructed three additional circuits (Table 1) and used these as negative controls to investigate whether the constructed circuits are functional.

Next we set about to test the transcriptional activity of the constructed malonyl-CoA sensors. *E. coli* cells transformed with different sensor constructs were grown in LB and induced with different concentrations of IPTG. Instantaneous eGFP expression and cell optical density were simultaneously recorded by a microplate reader (Figure 2). Interestingly enough, when we compared the results obtained in (a) T7-*lacO*-eGFP and (b) T7-*lacO*-fapO-eGFP (Figure 2), we found that the transcriptional activity of T7 promoter was decreased substantially compared with constructs in panels a and b. These results led us to the conclusion that the *B. subtilis* transcriptional factor FapR could possibly work as a regulatory repressor in the T7 expression system, either by directly interacting with *lacO* in Figure 2c or interacting with *fapO* in Figure 2d, although *lacO* is not the cognate DNA binding site for FapR. In addition to that, the hybrid promoters in Figure 2c,d exhibited biphasic kinetics that to a large extent, which could be attributed to the sequential expression of the two repressors inside the cell: the constitutive expression of the LacI repressor and IPTG-induced expression of FapR. In the first phase (*t < 300 min*), the transcriptional activity of this hybrid promoter was dominated by the LacI repressor, and as a result, we saw an increased promoter activity when we increased the level of IPTG; in the second phase (*t > 300 min*), the transcriptional activity of this hybrid promoter was dominated by the FapR repressor, and as a result we saw a decreased promoter activity when we increased the expression level of FapR by increasing IPTG.

From these data, it was still unclear how the transcriptional activities of these hybrid synthetic promoters would correlate with the level of malonyl-CoA inside the cell. Therefore, we decided to investigate how the phase I and phase II dynamic promoter activities would change at different levels of malonyl-CoA.

**Activity of T7-Based Malonyl-CoA Sensor in the Presence of Cerulenin.** Because malonyl-CoA is an active CoA-ester intermediate, extraction and detection of intracellular malonyl-CoA require efficient quenching and protection agents as well as complicated analytical techniques. It is impossible to titrate the malonyl-CoA level for each of the tested strains. To simplify this process, we attempted to use a chemical inhibitor, cerulenin, that blocks the *E. coli* native fatty acids pathway and as a result forces the cell to build up...
malonyl-CoA. Next we titrated the intracellular malonyl-CoA in E. coli BL21 by supplementing the culture medium with different concentrations of cerulenin. LC-MS results indicated that the level of malonyl-CoA that accumulated inside the cell was positively correlated with the level of cerulenin added in the culture medium (Figure S1, Supporting Information). Using cerulenin as means to control intracellular levels of malonyl-CoA, we set about to test how the constructed sensors would respond to malonyl-CoA.

We first tested the transcriptional activity of the four hybrid T7 promoters by adding different levels of cerulenin into the culture medium (Figures S2–S5, Supporting Information). We observed that eGFP expression linearly increased with time after a short time period (about 90 min) in all the tested constructs. With the expression of FapR, however, both sensor constructs T7-lacO-eGFP-T7-lacO-fapR (Figure S4, Supporting Information) and T7-lacO-fapO-eGFP-T7-lacO-fapR (Figure S5, Supporting Information) showed decreased transcriptional activity compared with the two control circuits (Figures

Table 1. Construction of Malonyl-CoA Sensor with Different Regulatory Architectures

<table>
<thead>
<tr>
<th>No.</th>
<th>Sensor name</th>
<th>fapO</th>
<th>eGFP</th>
<th>fapR</th>
<th>Diagram</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>lacO-fapO-eGFP</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
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<td>lacO-eGFP + lacO-fapR</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>![Diagram](T7-lacO-eGFP + T7-lacO-fapR)</td>
</tr>
<tr>
<td>IV</td>
<td>lacO-fapO-eGFP + lacO-fapR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>![Diagram](T7-lacO-fapO-eGFP + T7-lacO-fapR)</td>
</tr>
</tbody>
</table>

“The first two sensors were used as negative controls to test the functionality of the last two sensors.

Figure 2. Characterization of the transcriptional activity of hybrid T7 promoter incorporating the B. subtilis fapR and fapO regulatory elements in E. coli BL21 grown in LB and induced with IPTG: (a) BL21* transformed with T7-lacO-eGFP; (b) BL21* transformed with T7-lacO-fapO-eGFP; (c) BL21* transformed with T7-lacO-eGFP and T7-lacO-fapR; (d) BL21* transformed with T7-lacO-fapO-eGFP and T7-lacO-fapR.

suggesting that FapR can act as a repressor in this T7-based transcription system. It is important to note that even though \( \text{lacO} \) is not the cognate DNA binding site for FapR, we hypothesize that FapR can cross-communicate with \( \text{lacO} \) and repress eGFP expression in the sensor T7-lacO-eGFP-T7-lacO-fapR (Figure S4, Supporting Information). Interestingly, we also found that the transcriptional activity of the two sensor constructs (Figures S4 and S5, Supporting Information) was substantially increased when the concentration of cerulenin in the culture medium increased, indicating that malonyl-CoA is an upregulating effector in these two sensor systems. We found similar biphasic kinetics (Figures S4 and S5, Supporting Information) when cerulenin concentration was low (<25 \( \mu \text{M} \)). However, when the level of cerulenin was increased to 100 \( \mu \text{M} \), the biphasic kinetics completely disappeared, possibly because the increased concentration of malonyl-CoA antagonized most of the FapR, thus leaving the sensors subject to LacI control only.

**Phase I Kinetics of T7-Based Malonyl-CoA Sensor.**
Supplementation of chemical inhibitors tends to elicit complex phenotypes and can lead to unpredictable cellular transcriptional activity. Because the level of cerulenin also affected the transcriptional activity of the control circuits lacO-eGFP (Figure S2, Supporting Information) and lacO-fapO-eGFP (Figure S3, Supporting Information), the relative promoter activity fold change, a quantity that eliminates promoter activity change due to cerulenin, was a more reliable indicator to describe how the introduced fapO or FapR affected the transcriptional activity of these hybrid promoters. For this reason, we examined the relative transcriptional activity fold change in the four tested sensors (Figure 3).

With the introduction of fapO, the relative promoter activity fold change remained about 110% of the control circuit regardless of whether fapR was expressed (Figure 3, panel b vs a) at different levels of malonyl-CoA. This indicated that the introduced fapO did not affect the activity of the hybrid promoter. However, when fapR is expressed, the saturation relative fold change was less than one and ranged from 0.28 to 0.84 when the FapR-binding site (fapO) is present (Figure 3d) or not present (Figure 3c). This indicated that FapR can bind to fapO or lacO and repress the transcription of this hybrid T7 system. Moreover, the relative promoter activity fold change exhibited increased value as the level of intracellular malonyl-CoA was increased (Figure 3c,d). For example, when the intracellular level of malonyl-CoA was low (0.12 nmol/mgDW), the transcriptional activity of the sensor circuit lacO-eGFP-lacO-fapR showed about 27% of the transcriptional activity of the control circuit lacO-eGFP (Figure 3c). On the other hand, when cellular malonyl-CoA was high (1.06 nmol/mgDW), the transcriptional activity of the sensor circuit lacO-eGFP-lacO-fapR showed restored to about 85% of the transcriptional activity of the control circuits (Figure 3c). From the phase I kinetics, we concluded that FapR is a repressor for T7-based malonyl-CoA and cerulenin can be found in the Supporting Information, Figure S1.

**Phase II Kinetics of T7-Based Malonyl-CoA Sensor.**
The rate of fluorescence change normalized with cell density was used to characterize the phase II dynamic promoter
activity, summarized in Figure 4 for the two sensor constructs (sensor III and sensor IV). As shown in Figure 4, the second phase shows inhibition kinetics when the cellular malonyl-CoA level is low (<0.63 nmol/mgDW). For example, the promoter activity increased initially when the amount of FapR expressed in the cell was low (at low concentration of IPTG) but started declining when the amount of FapR expressed in the cell reached a critical concentration (at higher concentration of IPTG). Essentially this inhibition kinetics could be explained as the competitive binding of fapO/lacO between FapR and LacI repressor proteins. On the other hand, when the level of malonyl-CoA was increased to 1.06 nmol/mgDW, this inhibition kinetics completely disappeared (Figure 4) due to the inactivation of FapR by malonyl-CoA. Consistent with the first phase kinetics, we also observed that increased malonyl-CoA has led to increased promoter activity in both the sensor construct without fapO (Figure 4a) and the sensor construct with fapO (Figure 4b). Taken together, the phase II dynamic promoter activity further supports the conclusion that FapR can cross-communicate with the LacI repressor binding site lacO, and malonyl-CoA is an upregulating effector that can antagonize the repression effects exerted by FapR.

**Figure 4.** Phase II transcriptional activity of T7-based malonyl-CoA sensor at different levels of malonyl-CoA: (a) sensor construct without fapO; (b) sensor construct with fapO. Malonyl-CoA concentration (nmol/mgDW): 0.12 (▼); 0.22 (▲); 0.63 (●); 1.06 (■). The level of malonyl-CoA was titrated with addition of different levels of cerulenin. Quantitative relationship between malonyl-CoA and cerulenin can be found in the Supporting Information, Figure S1.

**Surface Plasmon Resonance Analysis of FapR–lacO Interaction.** In order to physically validate that FapR cross-communicates with lacO, we performed surface plasmon resonance (SPR) analysis with purified FapR protein and biotinylated synthetic oligos (lacO and fapO) as testing subjects. The obtained results presented in Figure 5 support our conjecture that FapR indeed cross-talks with lacO. For example, FapR and lacO interaction has relatively fast association kinetics and slow dissociation kinetics (Figure 5). With these data, we analyzed the binding affinity between FapR and lacO/fapO (Table 2). Interestingly, SPR data (Table 2) show that the binding between FapR and lacO is about 2.1-times stronger than the binding between FapR and fapO.

Initially we were concerned that the expression of FapR would be negatively autoregulated by itself since the fapO expression cassette was placed under the control of T7/lacO. However, in all our tested sensors, we did not observe the expected autoregulation. We believe the nonoccurrence of autoregulation could be ascribed to two reasons. First SPR results indicated that the lactose repressor protein LacI binds with lacO much more strongly than FapR binds with lacO, since LacI/lacO interaction has a much smaller equilibrium dissociation constant \( K_D = 4.1 \times 10^{-11} \text{ M} \) than that of FapR/lacO interaction \( K_D = 5.7 \times 10^{-8} \text{ M} \) (Table 2). This indicates that the expression of FapR is predominantly controlled by LacI/lacO interaction rather than the FapR/lacO autoregulation. Second we have used a very stable GFP (with half-life time more than 20 h) in our studies, and therefore we believe that we cannot capture the transient dynamic behavior (oscillation) of our sensors due to the much shorter time-scale of bacterial gene transcription (E. coli gene transcription has a time-scale of seconds to minutes).

**Deletion of Lactose Repressor Binding Site Abolishes Biphasic Kinetics.** One important goal of synthetic biology is to characterize and identify orthogonal molecular components that could function independently and predictably. For this purpose, extensive work has been done toward the modification of effector specificity, change of promoter dynamic response activity and minimization of cross-communication effects. A common problem associated with sensor-regulatory design is the interference between regulatory protein and its noncognate DNA-binding site. Such off-target cross-communication effect could ultimately lead to the failure or malfunction of designed circuits. In the present work, the hybrid promoter—regulator

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**Figure 5.** Sensorgrams of FapR–fapO (a) and FapR–lacO (b) interaction. Concentration of FapR from top to bottom: 250, 125, 63, and 32 nM. The black curves are the fitting curves using models from BIAevaluate 4.0.1.
(T7-FapR) exhibits complex biphase kinetics due to the interaction between the B. subtilis regulatory protein FapR and the lactose repressor binding site lacO. In order to eliminate this nonspecific binding, we removed the built-in LacI repressor binding site (lacO) from our sensor construct by site-directed mutagenesis. In the presence of lacO, eGFP expression exhibited malonyl-CoA-dependent parabolic biphase kinetics (Figure 6a); interestingly enough, upon removal of lacO, eGFP expression underwent malonyl-CoA-dependent linear kinetics (Figure 6b). When the malonyl-CoA level was increased from 0.12 to 1.06 nmol/mgDW, the relative promoter activity (the rate of fluorescence change with time, namely, the slope of the curve) was increased 5-fold in the sensor construct without lacO (Figure 6b), compared with only 2-fold change in the sensor construct with lacO (Figure 6a). These results demonstrate that elimination of the noncognate DNA binding site not only changed the dynamic behavior but also the dynamic response range in the constructed sensors. Potentially, sensors with simple dynamic behavior and broad dynamic response range will more preferentially be used as regulatory devices to control biological functions.

**Conclusions.** In conclusion, we have constructed a synthetic genetic circuit that correlates T7 promoter activity to two input signals, IPTG and malonyl-CoA. Specifically, the cis-regulatory element fapO and reporter gene eGFP were placed downstream of the T7 promoter, while the trans-regulator protein encoded by fapR was expressed by an independent T7 promoter. Expression of eGFP depends on both the inducer IPTG and malonyl-CoA. In addition, FapR has been identified as a repressor for the T7-based transcription system and the hybrid T7 promoter was upregulated by the intracellular levels of malonyl-CoA. Because of the sequential expression of the two repressors LacI and FapR, the constructed circuits exhibited biphase transcriptional activity. Both the phase I and phase II dynamic promoter activity were positively related to the level of malonyl-CoA inside the cell. Deletion of the lactose repressor binding site lacO led to a sensor construct that undergoes single phasic kinetics. The constructed malonyl-CoA sensors can be employed as control elements in order to modulate gene expression of biosynthetic pathways of important compounds that are of particular interest to the pharmaceutical and biofuel industries.

**METHODS**

**Materials and Molecular Agents.** Plasmid construction and DNA manipulations were performed following standard molecular cloning protocols. Strains and plasmids used in this study are listed in Table S1, Supporting Information. All PCR primers used for site-directed mutagenesis and gene amplification are listed in Table S2, Supporting Information. Plasmid maintenance and propagation were performed using E. coli DH5α strain. Site-directed mutagenesis was performed in E. coli BW27784 using QuickChange II site-directed mutagenesis kits from Agilent. The duet vectors pET-Duet and pCDF-Duet were purchased from Novagen. All clones were screened by restriction digestion analysis and verified by gene sequencing.

**T7-Based Malonyl-CoA Sensor Construction.** The fapO operator DNA sequence was attached with eGFP was synthesized by Integrated DNA Technologies (IDT). Detailed sequence and gene context information is provided in Table S3, Supporting Information. The gene encoding for eGFP was PCR amplified from pIDTBlue-fapO-eGFP using primers eGFP_Nco and eGFP_Hin. Next, NdeI/HinIII digested PCR products were inserted into the NeoI and HindIII site of pET-Duet to give construct pET-eGFP. To incorporate fapO into the regulatory region of pET-Duet, fapO-eGFP fragment was cut from pIDTBlue-fapO-eGFP using restriction enzymes NdeI and HindIII. Next, the NdeI/HindIII digested gene fragment was gel purified and inserted to the Xhol/HindIII site of pET-Duet to give plasmid pET-fapO-eGFP.

The gene encoding for FapR was codon-optimized and synthesized by Integrated DNA Technologies (IDT). Detailed sequence and gene context information are provided in Table S4, Supporting Information. The fapR gene fragment was cut from pIDTBlue-fapR with restriction enzymes NdeI/XhoI. This gene fragment was then gel purified and inserted to the NdeI and XhoI site of pET-eGFP and pET-fapO-eGFP to give construct pET-eGFP-fapR and pET-fapO-eGFP-fapR, respectively. NdeI/XhoI digested fapR fragment was also inserted between the NdeI and XhoI sites of pCDF-Duet to give pCDF-fapR. The fapR × 6His gene fragment was PCR amplified using the primer pairs fapR_NdeI and fapR_XhoI. Then, this fragment was digested with NdeI and XhoI, and the gel purified fragment was inserted into the NdeI and XhoI digested pETM6 vector to give pETM6-fapR × 6His. His-tagged FapR protein was purified by the Qiagen Ni-NTA Fast Start kit following manufacturer’s protocol.

**Deletion of lacO on the Sensor Construct.** The ePathBrick vector pETM6 was modified to contain one additional XhoI site in front of lacO by Quickchange II site-directed mutagenesis kits using primer pairs Xba_T7IF and Xba_T7IR. The XhoI single digested pETM6 was gel purified and self-ligated to give vector pETM9. To construct plasmid pETM9-eGFP, the eGFP fragment was PCR...
amplified by using primer pairs eGFP_Nde and eGFP_Kpn. The Ndel/KpnI digested gene fragment was ligated to Ndel/KpnI digested pETM9 to give plasmid pETM9-eGFP. To construct pETM9-fapO-eGFP, Nhel and HindIII digested pIDTBlue-fapo-eGFP was first inserted to the Nhel and HindIII digested pBAD24 to give plasmid pBAD24-fapo-eGFP. A KpnI site was then inserted to pBAD24-fapo-eGFP right before the HindIII site. Nhel and KpnI digested pBAD24-fapo-eGFP fragment was gel-purified and ligated to the XbaI and KpnI digested pETM9 to give construct pETM9-fapo-eGFP.

Assay of Sensor Activity. Fluorescence signal intensity was used to characterize the promoter activity among the engineered sensors. Host cell BL21 (DE3) transformed with different sensor plasmids was grown overnight in LB at 37 °C and 250 rpm agitation. The next morning, 10 mL of fresh LB was inoculated with 8% (v/v) overnight culture in 50 mL Corning tubes and grown at 37 °C, 250 rpm, for approximately 1 h (OD of 0.2 in 96 well plate). Subsequently, 240 μL of cell culture was transferred to a Greiner Bio-one 96-well fluorescence plate (Bio-Greiner, chimney black, flat clear bottom) using an Eppendorf multichannel pipet. Different amounts of IPTG were added to the cell culture to induce the expression of GFP, together with different amounts of cerulenin. The fluorescence plates were covered and sealed with parafilm to prevent any volume decrease due to evaporation. Cells were left to grow at 37 °C with shaking at 300 rpm on a benchtop plate shaker (Labnet VorTemp 56 shaker incubator). Cell optical density and expression of green fluorescence protein were simultaneously detected every 30 min on a Biorex 4 microplate reader. Optical density was read at 600 nm, and the excitation and emission wavelengths for eGFP were set at 485 ± 20 and 528 ± 20 nm, respectively. All experiments were performed in triplicate.

Quantitative Analysis of Malonyl-CoA. E. coli BL21 was preincubated into fresh LB media and left to grow at 37 °C overnight. The next morning, 10 mL of fresh LB was inoculated with 200 μL of overnight culture, and cultures were grown at 37 °C in a 50 mL corning tube for about 1 h until the OD reached 0.2. Different amounts of cerulenin (0, 5, 10, 15, 20, 25, 50, and 100 μM) were added to the culture, and the cells were left to grow at 37 °C for an additional 1 h. The cells were then chilled on ice, and the culture was centrifuged at 4500 rpm and 4 °C for 15 min. The supernatant was discarded and the cell pellet was resuspended in 1.0 mL of 6% perchloric acid to facilitate cell lysis. The lysed cell suspension was then neutralized with 0.3 mL of 3 M potassium carbonate and then neutralized with 0.3 mL of 3 M potassium carbonate and 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4) running buffer was injected over the flow cell of the SA chip at a flow rate of 20 μL/min. The successful immobilization was confirmed by the observation of a 790 to 1500 resonance unit (RU) increase in the sensor chip. The control flow cell (FC1) was prepared by 1 min injection with saturated biorb. Measurement of Interaction between DNA and FapR Using BIACore. The protein samples were diluted in HBS-EP buffer. Different dilutions of protein samples were injected at a flow rate of 30 μL/min. At the end of the sample injection, the same buffer was flowed over the sensor surface to facilitate dissociation. After a 3 min dissociation time, the sensor surface was regenerated by injecting with 30 μL of 2 M NaCl to get fully regenerated surface. The response was monitored as a function of time (sensogram) at 25 °C.

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**REFERENCES**


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