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Noise Propagation in Synthetic Gene Circuits for Metabolic Control

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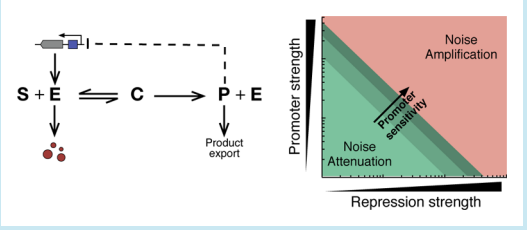
Supporting Information

ABSTRACT: Dynamic control of enzyme expression can be an effective strategy to engineer robust metabolic pathways. It allows a synthetic pathway to self-regulate in response to changes in bioreactor conditions or the metabolic state of the host. The implementation of this regulatory strategy requires gene circuits that couple metabolic signals with the genetic machinery, which is known to be noisy and one of the main sources of cell-to-cell variability. One of the unexplored design aspects of these circuits is the propagation of biochemical noise between enzyme expression and pathway activity. In this article, we quantify the impact of a synthetic feedback circuit on the noise in a metabolic product in order to propose design criteria to reduce cell-to-cell variability. We consider a stochastic model of a catalytic reaction under negative feedback from the product to enzyme expression. On the basis of stochastic simulations and analysis, we show that, depending on the repression strength and promoter strength, transcriptional repression of enzyme expression can amplify or attenuate the noise in the number of product molecules. We obtain analytic estimates for the metabolic noise as a function of the model parameters and show that noise amplification/attenuation is a structural property of the model. We derive an analytic condition on the parameters that lead to attenuation of metabolic noise, suggesting that a higher promoter sensitivity enlarges the parameter design space. In the theoretical case of a switch-like promoter, our analysis reveals that the ability of the circuit to attenuate noise is subject to a trade-off between the repression strength and promoter strength.



KEYWORDS: *dynamic metabolic engineering, genetic feedback circuits, biochemical noise, enzymatic reactions, promoter design, feedback control design*

Pathway engineering relies on heterologous enzymes that use the metabolic intermediates of a host as precursors for chemicals of industrial relevance. To date, pathway design uses a combination of computer-aided optimization and trial-and-error experimentation.[1,2] This is typically a lengthy process aimed at ruling out designs in which the chosen enzyme expression levels lead to lethal metabolic imbalances or impractically low yields.

A promising approach to optimize pathway design is to make enzyme expression dependent on the concentration of metabolic species.[3−5] This regulatory strategy is ubiquitous in natural pathways that need to sustain their homeostatic levels under changing environmental conditions.[6,7] In synthetic systems, pathway self-regulation can be achieved with gene circuits that dynamically up- or downregulate enzyme expression in response to changes in the level of a metabolite of interest. These feedback circuits can be designed to achieve *in vivo* self-regulation of enzyme levels that are compatible with the stoichiometric and kinetic constraints of the pathway under consideration.[8]

Following the first successful implementation of a feedback circuit for biofuel production,[5] in a recent work, we described some of the fundamental trade-offs in the design of a gene circuit for pathway control.[8] For a circuit based on a metabolite-responsive transcription factor, we identified constraints that prevent metabolite accumulation in terms of the promoter dynamic range and ribosomal binding site strengths. Other works have addressed a number of alternative circuit designs, including a quorum-sensing mechanism coupled with a genetic toggle switch[9,10] and the dynamic regulation of biofuel efflux pumps.[11] Altogether, this body of theoretical work has started to reveal the potential of gene circuits for pathway control, but we still lack a comprehensive understanding of how circuit design affects metabolic performance.

A design aspect that has been overlooked so far is the propagation of biochemical noise between enzyme expression and metabolic pathways. Genetic processes such as transcriptional and translational initiation typically depend on molecules that appear in low numbers and therefore are inherently noisy.[12,13] As a consequence, individual cells in an isogenic population can reach vastly different expression levels of the same protein. In natural systems, noise regulation is vital because of its decisive role in a number of cellular responses, including cell differentiation, DNA mutation, and cell death.[14] Cells typically need to attenuate noise to execute their functions

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accurately, but some cellular phenotypes have also been shown to depend on stochastic decisions.[15] In engineered pathways, however, uncontrolled cell-to-cell variability can be detrimental to metabolic production goals and thus synthetic gene circuits should ideally achieve low noise levels.

Stochastic effects in metabolic pathways were first studied in[16] considering fluctuations in substrates and a constant abundance of catalytic enzymes. The propagation of noise between enzyme expression and metabolism, however, has been typically neglected on the basis that high metabolite counts average out stochastic effects. As a consequence, nearly all studies on biochemical noise have focused on gene expression in isolation from metabolism (two exceptions are a simulation study on the tryptophan operon in *B. subtilis*[17] and a noise sensitivity method applicable to metabolic reactions[18]). Although this assumption may be justified in natural metabolic networks, engineered pathways can operate in low-yield regimes where the effects of noise become more apparent. Moreover, classical results in Control Engineering[19] indicate that the propagation of noise in feedback systems can be shaped by an appropriate design of the regulatory feedback. The effect of feedback on gene expression noise was first demonstrated in two seminal works[20,21] that suggested negative autoregulation as a mechanism for attenuation of stochastic fluctuations in protein numbers. Further theoretical[22−26] and experimental[27] research has revealed a more intricate relation between negative feedback and noise, indicating that noise can be attenuated or amplified depending on the feedback strength.

In this article, we quantify the impact of transcriptional repression on the noise in metabolic product in order to identify design criteria to reduce the impact of genetic noise in synthetic pathways. We focus on a metabolic reaction coupled with the feedback circuit shown in Figure 1. (The reaction

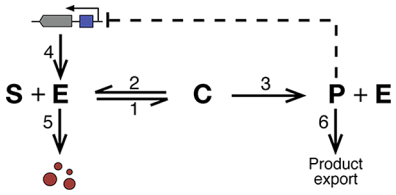

Figure 1. Stochastic model for a metabolic reaction under transcriptional repression from the product. Species S, E, C, and P represent the substrate, enzyme, substrate−enzyme complex, and product, respectively; the individual reactions are described in Table 2.

propensities and parameters for this model are shown in Table 1.) In this system, the metabolic product represses the expression of the catalytic enzyme via a transcriptional circuit. A possible implementation of this circuit is via a transcription factor that controls the expression of a metabolic gene in response to the level of product.[5] We base our study on a stochastic model for the feedback circuit in Figure 1 and a combination of stochastic analysis and simulations. The model accounts for catalysis, product-dependent enzyme expression, enzyme degradation, and export of the metabolic product. We use a lumped model for gene regulation parametrized in terms of the promoter strength, promoter sensitivity, and repression strength, all of which are considered as design parameters and can potentially affect the levels of metabolic noise.

One of the key challenges of genetic−metabolic systems is that, owing to the wide separation of time scales between enzyme expression and enzyme kinetics, stochastic simulations are impractically slow to run. We overcame this problem by using a fast simulation algorithm tailored for genetic−metabolic systems.[28,29] We computed histograms of the stationary distributions of product and enzyme molecule numbers for different circuit designs. Using the squared coefficient of variation of these distributions as a measure of noise,[13,22,23,30] we quantified the effect of feedback parameters on noise and compared it to the case of constitutive enzyme expression. The simulations suggest that (a) a weak repression or weak promoter effectively attenuate noise but above critical strengths, the circuit leads to amplification of noise and (b) the promoter sensitivity enhances noise attenuation and amplification. Motivated by these numerical observations, we used the linear noise approximation[31] to obtain analytic estimates for the metabolic noise as a function of the design parameters. We analytically proved the validity of the attenuation/amplification phenomenon for these estimates and obtained a simple condition on the design parameters for noise attenuation. In the limit case of a switch-like promoter, we show that attenuation of metabolic noise is subject to a trade-off between the promoter strength and repression strength, in which attenuation can be achieved by strong promoters that are weakly repressed or, conversely, by weak promoters under strong repression.

RESULTS AND DISCUSSION

Stochastic Model for an Enzymatic Reaction under Transcriptional Feedback Repression. We consider a metabolic reaction that converts a substrate, S, into a product, P, via an enzyme, E, that is repressed by the product. The model system is shown in Figure 1, including the binding and dissociation of the substrate and enzyme into a complex, C, the degradation of enzyme molecules, and the export of product outside the cell. This type of circuit has been implemented, for example, with metabolite-responsive transcription factors[5] (TF). We assume that the number of substrate molecules is constant, accounting for scenarios in which the substrate is an extracellular nutrient pool consumed by a low-density cell population. Note that with this assumption we exclude the case in which the catalytic reaction eventually depletes the substrate and reaches a nil equilibrium.

If we define $n = (n_E, n_C, n_P)^T$ as the vector of molecule numbers and assume that the probability that two molecules bind depends only on the current state of the system (and not on its history), then the evolution of the probability distribution $P(n,t)$ satisfies the chemical master equation[31]

$$\frac{\partial P(n,t)}{\partial t} = \sum_{i=1}^{6} a_i(n - R_i)P(n - R_i,t) - \sum_{i=1}^{6} a_i(n)P(n,t) \tag{1}$$

where R_i is the ith column of the matrix

$$R = \begin{bmatrix} -1 & 1 & 1 & 1 & -1 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \end{bmatrix} \tag{2}$$

The quantities a_i in the master equation are the reaction propensities, so $a_i \times dt$ is the probability that reaction i occurs in an infinitesimal time interval, dt. With the exception of the gene regulatory interaction, the propensities in Figure 1 correspond to mass action kinetics (Table 2). We model gene regulation via a sigmoidal function that describes the

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input−output characteristic of the promoter in response to the metabolic product

$$a_4 = \frac{\beta}{1 + (n_P/\alpha)^h} \quad (3)$$

The model for a_4 is a lumped description of the processes that interface the metabolic product with enzyme expression. In particular, when using a metabolite-responsive TF, the propensity a_4 includes both the TF-product binding and the TF-mediated repression of promoter activity. We parametrize the enzyme expression model in terms of three design parameters:

(1) Promoter strength. β represents the maximal activity of the promoter in the absence of product molecules.
(2) Promoter sensitivity. h is a Hill coefficient describing the sensitivity of the promoter to changes in the product around the threshold α.
(3) Repression strength. α is the repression threshold (in numbers of product molecules) required to reduce the probability of enzyme production by 50%. The inverse of the threshold, $1/\alpha$, corresponds to the strength of the feedback repression.

The promoter strength can potentially be modified, for example, via the RNA polymerase binding sites,[32] whereas promoter sensitivity has been successfully manipulated with protein sequestration mechanisms.[33] The manipulation of the repression strength will largely depend on the specific biochemical mechanism used to couple the metabolic product with enzyme expression (for example, when using a metabolite-responsive TF, the repression strength can be modified via the TF-product binding affinity).

We quantify the noise in the number of product molecules by the squared coefficient of variation of its stationary distribution. We define the metabolic noise as $\eta_P^2 = \sigma_P^2/\mu_P^2$, with μ_P and σ_P being the mean and standard deviation of the marginal stationary distribution of n_P. The stationary distribution is a time-independent solution of the master equation and describes the statistics of molecule numbers after transient effects have vanished. Analytic solutions for the stationary distribution can be obtained only in few special cases,[34,35] and, in general, the most common alternative is to compute η_P^2 from histograms of sufficiently long realizations of the stochastic model.

The noise η_P^2 is a dimensionless quantity that has proven to be useful to quantify the size of stochastic fluctuations in biochemical systems.[13,22,23,30] To quantify the effect of transcriptional regulation on the metabolic noise, we also define the relative noise $\tilde{\eta}_P^2$ as the noise in the product normalized to the noise generated by a constitutive promoter with the same strength. For a given promoter strength β, the relative noise is $\tilde{\eta}_P^2 = \eta_P^2/\tilde{\eta}_{P\,\mathrm{unreg}}^2$, with $\eta_{P\,\mathrm{unreg}}^2$ being the noise in the product obtained with a constant propensity for enzyme expression ($a_4 = \beta$). The relative noise has been previously used to quantify stochasticity in gene expression;[26] here, we use it to determine whether transcriptional regulation amplifies or attenuates metabolic noise with respect to constitutive enzyme expression by checking the condition $\tilde{\eta}_P^2 > 1$ (amplification) or $\tilde{\eta}_P^2 < 1$ (attenuation).

Fast Simulations of the Stochastic Model. Gillespie's algorithm[36] is the classic approach for stochastic simulation of chemical systems. Although the algorithm gives statistically exact simulations, in the case of enzymatic reactions it entails impractically long simulation times (on the order of tens of hours per run) that prevent the quantification of metabolic noise as a function of the circuit design parameters. This is because the binding and dissociation reactions (labeled 1 and 2 in Figure 1, respectively) occur on a much shorter time scale than the product-forming reaction (reaction 3 in Figure 1); therefore, the algorithm needs to simulate thousands of binding and dissociation reactions per each birth of a product molecule. The inclusion of enzyme expression in our model aggravates this computational limitation, as gene expression dynamics occur on an even slower time scale than catalytic conversion.

We used the slow-scale simulation algorithm[28] to overcome these computational problems and to compute approximate stationary distributions rapidly. The algorithm is based on a time scale separation, and in a previous work[29] we adapted it to account for metabolic reactions with constant substrate, product export/consumption, and enzyme expression and degradation. In Figure 2, we show one run of the adapted

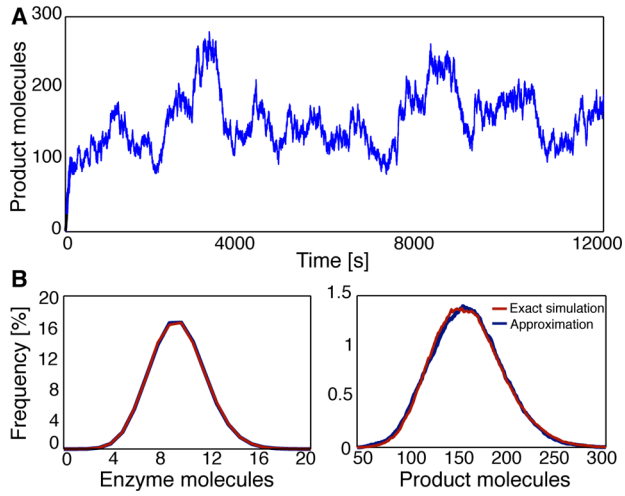

Figure 2. Fast stochastic simulations of the enzymatic reaction in Figure 1. (A) Time course of the number of product molecules computed with a fast simulation algorithm tailored for genetic−metabolic systems.[29] (B) Stationary distributions of species molecule numbers for the parameters in Table 2, with a repression threshold of $\alpha = 10$ molecules, promoter strength $\beta = 0.16$ molecules s^{-1}, and sensitivity $h = 1$.

algorithm, providing stationary distributions that are practically indistinguishable from those computed with Gillespie's algorithm. The approximate distributions can be computed up to 3 orders of magnitude faster than the exact simulations and thus the algorithm is suitable for exploring the effect of the circuit parameters on the metabolic noise.

We ran stochastic simulations of the feedback system for different combinations of repression strength, promoter sensitivity, and promoter strength. The results, summarized in Figure 3, indicate that

(1) The negative feedback reduces the mean number of product molecules and the variance of stochastic fluctuations with respect to a constitutive promoter. Note that for varying repression strengths and a fixed promoter strength (as in Figure 3A) the constitutive case is independent of α and corresponds to $1/\alpha = 0$, whereas for varying promoter strengths and a fixed repression strength (as in Figure 3B), the statistics of the

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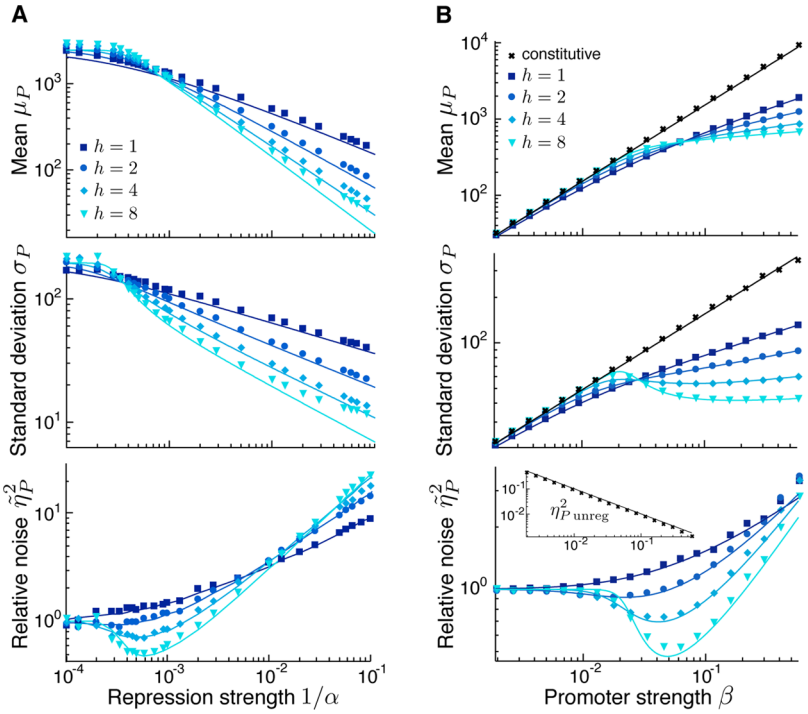

Figure 3. Statistics of the number of product molecules for different feedback parameters. (A) Fixed promoter strength (β = 0.16 molecules s^{-1}) with different combinations of repression strength and promoter sensitivity. (B) Fixed feedback strength (α = 500 molecules) with different combinations of promoter strength and sensitivity. The relative noise is the squared coefficient of the variation of the stationary product distribution, normalized to the squared coefficient of the variation of the distribution obtained with a constitutive promoter with the same strength, $\tilde{\eta}_P^2 = (\sigma_P/\mu_P)^2)/\tilde{\eta}_{P\,\mathrm{unreg}}^2$. The parameter ranges used in the stochastic simulations cover physiologically relevant mean product concentrations in *E. coli*[37] (~58 nM to 2 μM in panel A and ~50 nM to 15 μM in panel B). The product statistics obtained for a constitutive promoter as a function of the promoter strength are also shown in panel B. The markers are the values computed from stationary distributions obtained via fast stochastic simulations; the solid lines are the predictions of the linear noise approximation. All of the remaining parameters are reported in Table 2. The results for the enzyme molecule numbers are shown in Supporting Information Figure S1.

constitutive case depend on the promoter strength (represented as black crosses in Figure 3B).

(2) A stronger repression or a weaker promoter lead to a decrease in the mean number of product molecules. We observe a similar monotonic behavior in the product variance for stronger repression but not for weaker promoters.

(3) The relative noise $\tilde{\eta}_P^2$ displays a nonmonotonic behavior with respect to the repression strength and promoter strength. This suggests that weak repression or a weak promoter attenuate noise ($\tilde{\eta}_P^2 < 1$) but above a critical repression or promoter strength, the feedback can amplify noise ($\tilde{\eta}_P^2 > 1$). This phenomenon seems to appear only in the case of a Hill coefficient greater than 1 and, moreover, more sensitive promoters lead to a more pronounced effect on the relative noise. We observed similar trends in the stationary statistics of the number of enzyme molecules (see Supporting Information Figure S1).

Analytic Characterization of Metabolic Noise. To understand the effect of the repression strength and promoter sensitivity on the metabolic noise, we sought to obtain an estimate for η_P^2 as a function of the model parameters. We used the linear noise approximation[31] to compute estimates of the stationary distribution and its corresponding coefficient of variation (details are given in Methods A). This approximation assumes that the molecular fluctuations are small deviations around the mean and provides an analytic expression for a Gaussian approximation of their stationary distribution. As observed in Figure 3, the approximation predicts noise curves with the same nonmonotonic behavior observed in the stochastic simulations. This is expectable given the Gaussian-like distributions observed in the simulations (Figure 2B) and that the enzyme kinetic parameters satisfy the conditions that validate the linear noise approximation.[38] With a constant number of substrate molecules, the enzyme operates in a kinetic regime characterized by a constant turnover rate (i.e. a constant catalytic rate per unit of enzyme). For an enzyme with a turnover rate g (in units of s^{-1}), the relative metabolic noise predicted by the linear noise approximation is (details are given in Methods B)

$$\tilde{\eta}_P^2 = \frac{\eta_P^2}{\eta_{P\,\mathrm{unreg}}^2} = \frac{\beta}{k\mu_P} - \left(\frac{k_S + g}{k_S + k_6 + g}\right)\frac{\beta h \mu_P^h}{\beta\alpha^h + hk\mu_P^{h+1}} \quad (4)$$

where μ_P is the mean number of product molecules, k_S is the enzyme degradation rate constant, k_6 is the rate constant of product export, and $k = k_S k_6/g$. The formula in eq 4 shows the dependency of the relative metabolic noise on the mean number of product molecules μ_P. However, because the mean μ_P also depends on the model parameters but cannot be written as an explicit function of them, the expression for $\tilde{\eta}_P^2$ does not fully reveal the dependency of the noise on the design parameters. We used the expression in eq 4 to prove analytically that the relative noise is a nonmonotonic function of the repression strength and promoter strength (details are

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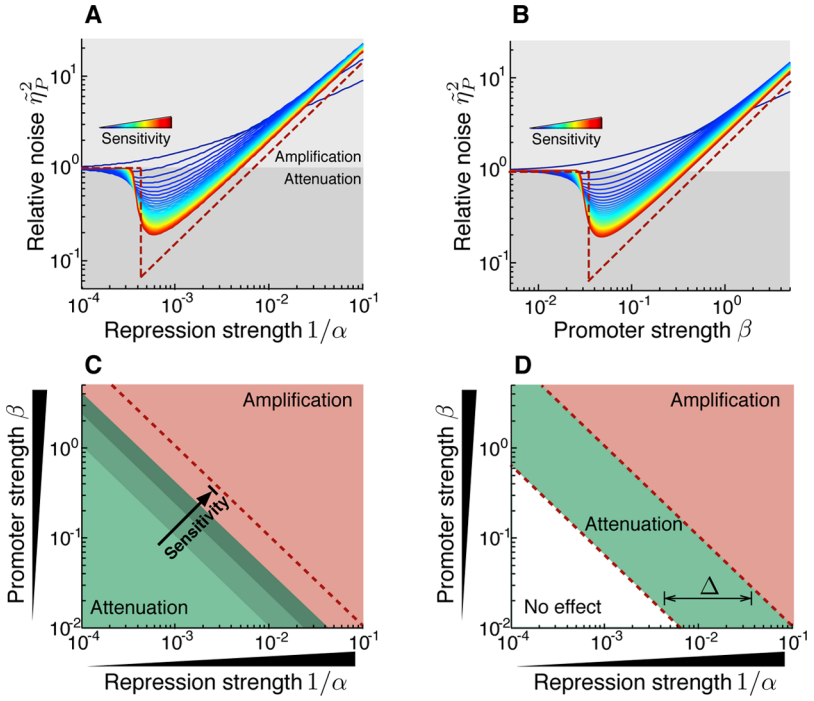

Figure 4. Attenuation and amplification of metabolic noise in the negative feedback circuit. (A) Relative metabolic noise $\tilde{\eta}_P^2$ for a fixed promoter strength (β = 0.16 molecules s^{-1}) and increasing repression strength and promoter sensitivity. (B) Relative metabolic noise $\tilde{\eta}_P^2$ for a fixed repression strength (α = 500 molecules) and increasing promoter strength and sensitivity. The curves are predictions of the linear noise approximation as given in eq 4, with the promoter sensitivity ranging from h = 1 to 40. The dashed red line corresponds to a switch-like promoter (the curve $\tilde{\eta}_{\text{limit}}^2$ shown in eq 7). (C) Parameter space for noise attenuation. The green area represents the combinations of repression and promoter strength that lead to noise attenuation. As shown in eq 5, in logarithmic scale, the boundary of the attenuation region is a line with slope −1. The attenuation region expands for increasing sensitivities (the plotted regions correspond to sensitivity h = {2,4,8}) and approaches the switch-like case (marked in red dashed line). (D) Parameter space for noise attenuation with a switch-like promoter. The attenuation band represents condition 8, and its width, Δ, is given by the expression in eq 9. In all panels, the remaining parameters were taken from Table 1.

given in Methods C and the Supporting Information). The analysis reveals that attenuation of metabolic noise appears when the Hill coefficient is $h > 1$, as suggested by the simulations in Figure 3, and indicates that it is a structural feature of the considered model for feedback repression.

The simulations in Figure 3 suggest that promoter sensitivity can enhance the attenuation and amplification of metabolic noise. We explored this dependency further by computing the predictions of the linear noise approximation for a more fine-grained and larger range of promoter sensitivities (Figure 4A,B). We found that an increased promoter sensitivity enlarges the region for noise attenuation. Detailed analysis of the estimate in eq 4 revealed that feedback repression attenuates noise when the repression strength $(1/\alpha)$ and promoter strength (β) satisfy the condition (details are given in Methods C and the Supporting Information)

$$\frac{\beta}{\alpha} < \frac{k}{w}\sqrt[h]{\frac{1-w}{w}} \tag{5}$$

where w is the constant

$$w = \frac{k_6}{k_5 + k_6 + g} + \frac{1}{h} \tag{6}$$

The condition in eq 5 describes all combinations of repression strength and promoter strength that lead to attenuation of metabolic noise. As illustrated in Figure 4C, this condition can be understood as a critical line in a logarithmic $(1/\alpha,\beta)$ parameter space. In addition, we observe that the constant w decreases in the sensitivity (h) and therefore more sensitive promoters tend to expand the range for noise attenuation.

In the theoretical case of a switch-like promoter, the noise curves approach a limit given by (details are given in Methods D)

$$\tilde{\eta}_{\text{limit}}^2 = \begin{cases} 1 & \text{for } \beta/\alpha < k, \\ \dfrac{\beta}{\alpha}\left(\dfrac{k_6}{k_5 + k_6 + g}\right) & \text{for } \beta/\alpha > k \end{cases} \tag{7}$$

The limit $\tilde{\eta}_{\text{limit}}^2$ is shown in red dashed line in Figure 4A,B, and its minimum value is the best possible noise attenuation the feedback can achieve. The condition for noise attenuation in the case of a switch-like promoter is (details are given in Methods D)

$$k < \frac{\beta}{\alpha} < k\left(\frac{k_5 + k_6 + g}{k_6}\right) \tag{8}$$

As shown in Figure 4D, the limit $\tilde{\eta}_{\text{limit}}^2$ can be understood as an attenuation band in a logarithmic $(1/\alpha,\beta)$ parameter space. The shape of the attenuation band (Figure 4D) suggests that for switch-like promoters noise attenuation is subject to a trade-off between the repression and promoter strengths. The width of the attenuation band is (details are given in Methods D)

$$\Delta = \log\left(\frac{k_5 + k_6 + g}{k_6}\right) \approx \log\left(1 + \frac{\tau_P g}{\ln 2}\right) \tag{9}$$

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Table 1. Some Enzymes Subject to Natural and Synthetic Transcriptional Regulation[a]

enzyme	natural (N) or synthetic (S) pathway	K_m
β-galactosidase	(N) lactose operon[39]	8.3 mM (allolactose)
PRA isomerase	(N) tryptophan operon[39]	4.7−7 μM
phosphoenolpyruvate synthetase	(S) lycopene biosynthesis[3]	83 μM (pyruvate)
IPP isomerase	(S) lycopene biosynthesis[3]	0.8−22.5 μM
alcohol dehydrogenase	(S) biofuel synthesis[5]	10.6 mM

[a]The K_m values are those reported in the BRENDA database[48] for *E. coli*; for enzymes with data for several substrates, the chosen one is indicated in brackets.

where $\tau_P = \ln 2/k_6$ is the half-life of the metabolic product. Note that in eq 9 we have used the fact that the time scale of enzyme degradation is much longer than the time scale of product export so that the parameters typically satisfy $k_5 \ll k_6$. The width of the attenuation band therefore depends on the interplay between the enzyme catalytic efficiency (via the turnover rate g) and the processes that catalyze the export of the metabolic product (via the product half-life τ_P). Note that, in particular, the formula for Δ suggests that noise attenuation seems to be negligible for inefficient enzymes.

Discussion. Genetic regulation is widespread in natural metabolic pathways, and numerous examples[6,7,39] demonstrate how cells can use transcriptional feedback to control their homeostatic levels robustly and to generate diverse phenotypes.[40] Despite their ubiquity in natural systems, the use of genetic circuits in engineered pathways is relatively new. This novel approach has been termed "dynamic metabolic engineering",[9] with successful case studies being the seminal work on lycopene production[3] and recent applications to biofuel and fatty acid synthesis.[5,41]

One of the biggest challenges in building these circuits is the poor availability of modular mechanisms that can interface metabolic species with gene expression. Some of the approaches explored to date include natural and engineered promoters[5,42] and metabolite-responsive riboswitches.[43] Even if those interface mechanisms were in place, however, we still have a limited understanding of how circuit parameters should be tuned to achieve a satisfactory performance. In this article, we focused on noise propagation, a commonly overlooked aspect of synthetic circuit design. Biochemical noise can have functional roles in natural systems,[14] but for metabolic production, we seek a low noise in metabolic fluxes in order to narrow the variability across a population of isogenic cells. We aimed at understanding the effect of feedback circuit parameters on the propagation of noise in a catalytic reaction in order to propose design criteria for noise attenuation.

We found that depending on the repression and promoter strength, the feedback circuit can attenuate or amplify the noise levels in the metabolic product. Mild repression or weak promoters have an attenuating effect, but above a critical strength, they lead to amplification of noise with respect to a constitutive promoter. Intuitively, for a stable feedback system, feedback repression reduces the fluctuations of the metabolic product by increasing enzyme expression when the product is low and by decreasing enzyme expression when the product is high. Our results corroborate this intuition because in all explored cases we observed that negative feedback reduces the variance of product and enzyme fluctuations. However, when looking at the size of the fluctuations relative to their mean (by the squared coefficient of variation), our results suggest a nonmonotonic relation between the feedback parameters and noise.

To understand the attenuation/amplification phenomenon further, we used the linear noise approximation[31] to show analytically that it is a structural feature of the considered model. A number of previous works on autoregulatory gene circuits[22−25,27] have established that negative feedback alone does not imply attenuation or amplification of protein noise; instead, product noise seems to behave nonmonotonically with respect to the repression strength. Our results suggest that a similar dependency underpins the propagation of noise between enzyme expression and a metabolic product and, moreover, that attenuation/amplification of noise appears also as a function of the promoter strength.

We found a condition on the circuit design parameters (the promoter strength, promoter sensitivity, and repression strength) that leads to attenuation of noise in the metabolic product. This condition, shown in eq 5 and Figure 4C, is a simple and intuitive description of how the design parameters affect the ability of the synthetic circuit to attenuate noise. In particular, it suggests that noise attenuation is possible only when the Hill coefficient of the promoter characteristic is $h > 1$ and that the parameter space for noise attenuation grows with the use of more sensitive promoters. Ultrasensitive regulation has been previously shown to improve flux adaptation in unbranched metabolic pathways[44] and to improve noise rejection in signaling cascades.[45] Our results suggest that ultrasensitivity is also beneficial for the attenuation of metabolic noise and, in particular, in the case of a switch-like promoter, noise attenuation is ultimately constrained by a trade-off between the repression strength and the promoter strength. As illustrated in Figure 4D, this trade-off means that noise attenuation can be achieved by either strong repression of a weak promoter or weak repression of a strong promoter.

We have previously shown that strong promoters in a feedback circuit can effectively compensate for disturbances in metabolic fluxes.[8] Our results on noise further indicate that promoter design must account for the interplay between the ability to dampen disturbances and the deleterious amplification of noise. These findings suggest that such design compromise can be alleviated by using the repression strength as a new design "knob" to fine-tune the balance between disturbance rejection and noise amplification.

Because natural pathways typically operate in regimes of high metabolite counts, it is commonly assumed that they average out stochastic effects from enzyme expression. In addition, it could be argued that evolution has shaped the natural regulation of metabolic fluxes in order to reduce the variability introduced by genetic noise. Synthetic pathways, in contrast, require the assembly of genetic parts that have not evolved together and have been manipulated via, for example, promoter engineering to control transcription, the design of ribosomal binding sites to tune the strength of translation,[46] and the use of degradation tags to control protein half-lives.[47] Our results

Table 2. Reaction Propensities and Parameters for the Model in Figure 1[a]

description	propensity	parameter
a_1: substrate−enzyme binding	$k_1 n_E n_S$	$k_1 = 1\ s^{-1}\ molecule^{-1}$
a_2: substrate−enzyme dissociation	$k_2 n_C$	$k_2 = 28\,300\ s^{-1}$
a_3: product formation	$k_3 n_C$	$k_3 = 3.2\ s^{-1}$
a_4: transcriptional repression	$\beta/(1 + (n_P/\alpha)^h)$	
a_5: enzyme degradation	$k_5 n_E$	$k_5 = 10^{-3}\ s^{-1}$
a_6: product export	$k_6 n_P$	$k_6 = 2 \times 10^{-2}\ s^{-1}$

[a]In the stochastic model, species are given in the number of molecules and time is given in seconds. For an *E. coli* volume of $V = 10^{-15}$ L,[53] the kinetic parameters correspond to an enzyme with $k_{cat} = 3.2\ s^{-1}$ and $K_m = (N_A V)^{-1}(k_2 + k_3)/k_1 = 47\ \mu M$, with $N_A = 6.022 \times 10^{23}\ mol^{-1}$, being the Avogadro constant. The degradation and export rate constants, k_S and k_6, were chosen to reflect a 20-fold difference between the product and enzyme half-lives. The number of substrate molecules was fixed to $n_S = 3000$. The specific values for the regulatory parameters α, β, and h are reported in each figure.

indicate that these interventions may significantly shape the propagation of noise between the genetic and metabolic layers. Moreover, the effects of noise may become even more apparent in heterologous pathways operating in low-yield regimes, where the stochastic fluctuations of metabolites can be significant as compared to their average concentrations.

We have discussed our results in terms of design parameters that can typically be tuned with current experimental techniques while leaving the enzyme kinetics fixed. However, the noise properties of a metabolic reaction also depend on the kinetic parameters of its enzyme. As illustrated in Table 1, kinetic parameters can vary across several orders of magnitude for different enzymes and organisms. They are also subject to large uncertainty because they are estimated from *in vitro* assays that do not necessarily represent their *in vivo* kinetics. As a consequence, the design of a feedback circuit for a particular metabolic reaction may require a case-by-case analysis to determine whether the effect of noise is an important or a negligible aspect to be considered in the design.

We have deliberately used a lumped model for gene expression that allowed for a detailed computational and mathematical analysis of the circuit. The cost of this approximation is the loss of the biochemical mechanisms involved in the regulatory circuit. These cannot be neglected if the design requires fine-tuning of the repression strength, as the way to do so will largely depend on the specific biochemistry used to interface the pathway with the genetic machinery. Mechanistic models may also be important for studying the effect of transcriptional bursting.[49] Proteome and transcriptome data in *E. coli* suggests that bursting may be significant for some metabolic enzymes,[50] and it is unclear how enzyme bursts propagate through an enzymatic reaction. The complexity of detailed mechanistic models leads to significant computational and mathematical challenges that will require new tailored approaches to quantify stochasticity in genetic−metabolic systems. A number of approaches hold promise in this regard, including extensions to the linear noise approximation that exploit the separation of time scales[51] and simulation algorithms tailored for multiscale biochemical systems.[52]

In this work, we focused on the properties of intrinsic noise in metabolic reactions, that is, the noise coming from the inherent stochasticity of molecular interactions. It is widely accepted that gene expression noise can be ascribed to both intrinsic and extrinsic sources.[30] Extrinsic noise stems from variability in components of the genetic machinery (such as polymerases and σ factors) and environmental effects such as variability in extracellular substrates. The effect of stochastic fluctuations in substrate abundance has been explored previously[16] for metabolic pathways with constant enzyme concentrations. However, further work needs to be done to establish how synthetic feedback circuits affect both intrinsic and extrinsic stochasticity in metabolic pathways. This and other open questions can be addressed with model-based approaches as the one used here, which can provide valuable insights into the design of gene circuits for metabolic engineering.

METHODS

A. Linear Noise Approximation. Let $dc/dt = Nv(c)$ be the deterministic differential equation model corresponding to a stochastic chemical system, where $c = n/V$ is the vector of species concentrations (in molecules per liter) in a volume V, N is the stoichiometric matrix, and $v(c)$ is the vector of macroscopic reaction rates. Assuming that the mean molecule numbers match the steady-state concentration, $\mu = V\bar{c}$ with $Nv(\bar{c}) = 0$, and that the fluctuations are small around the mean, the stationary distribution $P(n)$ can be approximated by a multivariate Gaussian $\mathcal{N}(\mu,\Sigma)$ with a covariance matrix satisfying a Lyapunov equation[31]

$$A\Sigma + \Sigma A^T + VBB^T = 0 \tag{10}$$

where $A = N\,\partial v/\partial c|_{c=\bar{c}}$ is the Jacobian of the rate vector and $BB^T = N\mathrm{diag}\{v_i\}N^T$.

B. Derivation of the Metabolic Noise. To obtain analytic expressions for the noise in the metabolic product, we start from a deterministic ODE model for the system in Figure 1

$$\frac{dc_P}{dt} = gc_E - k_6 c_P \tag{11}$$

$$\frac{dc_E}{dt} = f(c_P) - k_S c_E \tag{12}$$

where $c_x = n_x/V$ is the concentration of species x (in molecules per liter), and g is the enzyme turnover rate (in units of s^{-1}) for a constant number of substrate molecules. The function f is the rate of enzyme synthesis, which, by comparison with propensity a_4, can be written as $f(c_P) = \beta_c(1 + (c_P/\alpha_c)^h)$, with parameters $\beta_c = \beta/V$ and $\alpha_c = \alpha/V$. The steady-state product concentration is the solution of the equation

$$f(\bar{c}_P) = k\bar{c}_P \tag{13}$$

with $k = k_S k_6/g$. It can be numerically checked that the steady-state product concentration of the model in eqs 11 and 12 satisfies the key assumption behind the linear noise approximation $\bar{c}_P \approx \mu_P/V$, where μ_P is the mean number of product molecules obtained via stochastic simulations.

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From the deterministic model in eqs 11 and 12, the stoichiometric matrix and the vector of macroscopic reaction rates are

$$N = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}, \quad v = [g\bar{c}_E \quad k_6\bar{c}_P \quad f(\bar{c}_P) \quad k_5\bar{c}_E]^T \tag{14}$$

To compute the metabolic noise, we substitute N and v into eq 10 and analytically solve for the covariance matrix

$$\Sigma = \begin{bmatrix} \sigma_{c_P}^2 & \sigma_{c_P c_E} \\ \sigma_{c_P c_E} & \sigma_{c_E}^2 \end{bmatrix} \tag{15}$$

where $\sigma_{c_P}^2$ and $\sigma_{c_E}^2$ are the variances of the concentrations of product and enzyme, respectively, and $\sigma_{c_P c_E}$ is their covariance. The metabolic noise can then be obtained as the ratio $\eta_P^2 = (\sigma_{c_P}/\bar{c}_P)^2$, with $\bar{c}_P$ given by the solution of eq 13. After algebraic manipulations (details omitted for brevity), we obtain an expression for the metabolic noise

$$\eta_P^2 = \frac{1}{\mu_P}\frac{k_6}{k_5 + k_6}\left(1 + \frac{k_g}{k - f'(\bar{c}_P)}\right) \tag{16}$$

where $f'(\bar{c}_P) = \partial f/\partial c_P|_{c_P=\bar{c}_P}$ and $k_g = k_5(k_5 + g)/g$. From the definition of $f(c_P)$ it follows that $f'(\bar{c}_P) = -hk^2\bar{c}_P^{h+1}/(\beta_c\alpha_c^h)$, which after substituting in eq 16 and rearranging terms, leads to

$$\eta_P^2 = \frac{1}{\mu_P}\left(1 + \frac{g}{k_5 + k_6}\right) - \left(\frac{k_5 + g}{k_5 + k_6}\right)\frac{hk\mu_P^h}{\beta\alpha^h + hk\mu_P^{h+1}} \tag{17}$$

The noise for the case of constitutive enzyme expression can be computed by taking the limit $\alpha \to \infty$ in eq 17

$$\eta_{P\,\mathrm{unreg}}^2 = \frac{1}{\mu_{P\,\mathrm{unreg}}}\left(1 + \frac{g}{k_5 + k_6}\right) \tag{18}$$

where $\mu_{P\,\mathrm{unreg}}$ is the mean product number obtained with a constitutive promoter. Taking the limit $\alpha \to \infty$ in the steady-state equation in 13, we get $\mu_{P\,\mathrm{unreg}} = \beta/k$, which, after substitution in eq 18, gives

$$\eta_{P\,\mathrm{unreg}}^2 = \frac{k}{\beta}\left(1 + \frac{g}{k_5 + k_6}\right) \tag{19}$$

The final expression for $\tilde{\eta}_P^2$ in eq 4 can obtained as the ratio of eqs 17 and 19.

C. Attenuation and Amplification of Metabolic Noise. We sought to validate analytically the behavior of the relative noise $\tilde{\eta}_P^2$ suggested by the simulation results in Figure 3. On the basis of the estimate of the linear noise approximation given in eq 4, we proved that (a) $\tilde{\eta}_P^2 \approx 1$ for small $1/\alpha$ or small β, (b) $\tilde{\eta}_P^2 > 1$ when $1/\alpha$ or β are large, and (c) $\tilde{\eta}_P^2 < 1$ if and only if the parameters satisfy the condition in eq 5.

Note that because the mean μ_P depends on all the parameters of the model (through the steady-state equation in 13, which does not have an analytic solution) it is not straightforward to use the formula in eq 4 to draw conclusions on how $\tilde{\eta}_P^2$ behaves as a function of the parameters. We proved (a) and (b) by computing asymptotic expressions for $\tilde{\eta}_P^2$ (in particular, we showed that $\log \tilde{\eta}_P^2$ grows linearly, with slope $h/(h + 1)$, in $\log 1/\alpha$ and $\log \beta$ when these parameters are sufficiently large). We proved (c) by algebraic manipulations of the inequality $\tilde{\eta}_P^2 < 1$. The detailed calculations are presented in the Supporting Information.

D. Theoretical Limit of the Metabolic Noise for Switch-Like Promoters. To compute the limit expression in eq 7, we note that when $h \to \infty$ the solution of the steady-state equation in 13 is

$$\mu_P = \begin{cases} \beta/\alpha & \text{for } \beta/\alpha < k, \\ \alpha & \text{for } \beta/\alpha > k \end{cases} \tag{20}$$

The expression for $\tilde{\eta}_{\mathrm{limit}}^2$ can then be obtained by substituting eq 20 into the estimate for $\tilde{\eta}_P^2$ in eq 4 and taking the limit $h \to \infty$. The condition for noise attenuation in eq 8 can be obtained by substituting eq 7 into the inequality $\tilde{\eta}_{\mathrm{limit}}^2 < 1$. The width of the attenuation band Δ can be obtained by applying the logarithm to the width of the range in eq 8.

■ ASSOCIATED CONTENT

Supporting Information

Detailed calculations for Methods C and statistics for the number of enzyme molecules. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

D.A.O. designed the research and developed the analytic results. J.-B.L. and D.A.O. produced the simulations. D.A.O., J.-B.L., and G.-B.V.S. analyzed the results.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Stephanopoulos, G., Aristidou, A., and Nielsen, J. *Metabolic Engineering: Principles and Methodologies*, Academic Press, San Diego, CA, 1998.

(2) Burgard, A. P., Pharkya, P., and Maranas, C. D. (2003) Optknock: A bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. *Biotechnol. Bioeng. 84*, 647−657.

(3) Farmer, W. R., and Liao, J. C. (2000) Improving lycopene production in *E. coli* by engineering metabolic control. *Nat. Biotechnol. 18*, 533−537.

(4) Holtz, W. J., and Keasling, J. D. (2010) Engineering static and dynamic control of synthetic pathways. *Cell 140*, 19−23.

(5) Zhang, F., Carothers, J. M., and Keasling, J. D. (2012) Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. *Nat. Biotechnol. 30*, 354−359.

(6) Zaslaver, A., Mayo, A., Rosenberg, R., Bashkin, P., Sberro, H., Tsalyuk, M., Surette, M., and Alon, U. (2004) Just-in-time transcription program in metabolic pathways. *Nat. Genet. 36*, 486−491.
(7) Kotte, O., Zaugg, J. B., and Heinemann, M. (2010) Bacterial adaptation through distributed sensing of metabolic fluxes. *Mol. Syst. Biol. 6*, 355-1−355-9.
(8) Oyarzún, D. A., and Stan, G.-B. V. (2013) Synthetic gene circuits for metabolic control: design trade-offs and constraints. *J. R. Soc., Interface 10*, 20120671.
(9) Anesiadis, N., Cluett, W. R., and Mahadevan, R. (2008) Dynamic metabolic engineering for increasing bioprocess productivity. *Metab. Eng. 10*, 255−266.
(10) Anesiadis, N., Kobayashi, H., Cluett, W. R., and Mahadevan, R. (2013) Analysis and design of a genetic circuit for dynamic metabolic engineering. *ACS Synth. Biol. 2*, 442−452.
(11) Dunlop, M. J., Keasling, J. D., and Mukhopadhyay, A. (2010) A model for improving microbial biofuel production using a synthetic feedback loop. *Syst. Biol. Synth. Biol. 4*, 95−104.
(12) Elowitz, M. B., Levine, A. J., Siggia, E. D., and Swain, P. S. (2002) Stochastic gene expression in a single cell. *Science 297*, 1183−1186.
(13) Paulsson, J. (2005) Models of stochastic gene expression. *Phys. Life Rev. 2*, 157−175.
(14) Rao, C. V., Wolf, D. M., and Arkin, A. P. (2002) Control, exploitation and tolerance of intracellular noise. *Nature 420*, 231−237.
(15) Raj, A., and van Oudenaarden, A. (2008) Nature, nurture, or chance: Stochastic gene expression and its consequences. *Cell 135*, 216−226.
(16) Levine, E., and Hwa, T. (2007) Stochastic fluctuations in metabolic pathways. *Proc. Natl. Acad. Sci. U.S.A. 104*, 9224−9229.
(17) Zamora-Chimal, C., Santillán, M., and Rodrguez-González, J. (2012) Influence of the feedback loops in the trp operon of *B. subtilis* on the system dynamic response and noise amplitude. *J. Theor. Biol. 310*, 119−131.
(18) Kim, K. H., and Sauro, H. M. (2012) Adjusting phenotypes by noise control. *PLoS Comput. Biol. 8*, e1002344-1−e1002344-14.
(19) Ogata, K. *Modern Control Engineering*, 5th ed., Prentice Hall, Boston, MA, 2010.
(20) Becskei, A., and Serrano, L. (2000) Engineering stability in gene networks by autoregulation. *Nature 405*, 590−593.
(21) Thattai, M., and van Oudenaarden, A. (2001) Intrinsic noise in gene regulatory networks. *Proc. Natl. Acad. Sci. U.S.A. 98*, 8614−8619.
(22) Shahrezaei, V., Ollivier, J. F., and Swain, P. S. (2008) Colored extrinsic fluctuations and stochastic gene expression. *Mol. Syst. Biol. 4*, 196-1−196-9.
(23) Singh, A., and Hespanha, J. P. (2009) Optimal feedback strength for noise suppression in autoregulatory gene networks. *Biophys. J. 96*, 4013−4023.
(24) Bruggeman, F. J., Blüthgen, N., and Westerhoff, H. V. (2009) Noise management by molecular networks. *PLoS Comput. Biol. 5*, e1000506-1−e1000506-11.
(25) Wang, L., Xin, J., and Nie, Q. (2010) A critical quantity for noise attenuation in feedback systems. *PLoS Comput. Biol. 6*, e1000764-1−e1000764-17.
(26) Voliotis, M., and Bowsher, C. G. (2012) The magnitude and colour of noise in genetic negative feedback systems. *Nucleic Acids Res. 40*, 7084−7095.
(27) Dublanche, Y., Michalodimitrakis, K., Kümmerer, N., Foglierini, M., and Serrano, L. (2006) Noise in transcription negative feedback loops: simulation and experimental analysis. *Mol. Syst. Biol. 2*, 41-1−41-12.
(28) Cao, Y., Gillespie, D., and Petzold, L. (2005) Accelerated stochastic simulation of the stiff enzyme-substrate reaction. *J. Chem. Phys. 123*, 144917.
(29) Lugagne, J.-B., Oyarzún, D. A., and Stan, G.-B. V. (2013) Stochastic simulation of enzymatic reactions under transcriptional feedback regulation. *Proc. Eur. Control Conf.*, 3646−3651.
(30) Swain, P. S., Elowitz, M. B., and Siggia, E. D. (2002) Intrinsic and extrinsic contributions to stochasticity in gene expression. *Proc. Natl. Acad. Sci. U.S.A. 99*, 12795−12800.
(31) van Kampen, N. G. *Stochastic Processes in Physics and Chemistry*, 3rd ed., Elsevier, London, 2007.
(32) Brewster, R. C., Jones, D. L., and Phillips, R. (2012) Tuning promoter strength through RNA polymerase binding site design in *E. coli*. *PLoS Comput. Biol. 8*, e1002811-1−e1002811-10.
(33) Buchler, N. E., and Cross, F. R. (2009) Protein sequestration generates a flexible ultrasensitive response in a genetic network. *Mol. Syst. Biol. 5*, 272-1−272-7.
(34) Jahnke, T., and Huisinga, W. (2007) Solving the chemical master equation for monomolecular reaction systems analytically. *J. Math. Biol. 54*, 1−26.
(35) Grima, R., Schmidt, D. R., and Newman, T. J. (2012) Steady-state fluctuations of a genetic feedback loop: An exact solution. *J. Chem. Phys. 137*, 035104-1−035104-13.
(36) Gillespie, D. (1977) Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem. 81*, 2340−2361.
(37) Alberts, B., Wilson, J., Johnson, A., Hunt, J., Raff, M., and Roberts, K. *Molecular Biology of the Cell*, 5th ed., Garland Science, New York, 2007.
(38) Thomas, P., Straube, A. V., and Grima, R. (2011) Limitations of the stochastic quasi-steady-state approximation in open biochemical reaction networks. *J. Chem. Phys. 135*, 181103-1−181103-4.
(39) Jacob, F., and Monod, J. (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol. 3*, 318−356.
(40) Oyarzún, D. A., Chaves, M., and Hoff-Hoffmeyer-Zlotnik, M. (2012) Multistability and oscillations in genetic control of metabolism. *J. Theor. Biol. 295*, 139−153.
(41) Liu, D., Xiao, Y., Evans, B., and Zhang, F. (2013) Negative feedback regulation of fatty acid production based on a malonyl-CoA sensor-actuator. *ACS Synth. Biol.*, DOI: 10.1021/sb400158w.
(42) Michener, J. K., Thodey, K., Liang, J. C., and Smolke, C. D. (2012) Applications of genetically-encoded biosensors for the construction and control of biosynthetic pathways. *Metab. Eng. 14*, 212−222.
(43) Winkler, W. C., Nahvi, A., Roth, A., Collins, J. A., and Breaker, R. R. (2004) Control of gene expression by a natural metabolite-responsive ribozyme. *Nature 428*, 281−286.
(44) Oyarzún, D. A., and Stan, G.-B. (2012) Design tradeoffs in a synthetic gene control circuit for metabolic networks. *Proc. 31st Am. Control Conf.*, 2743−2748.
(45) Thattai, M., and van Oudenaarden, A. (2002) Attenuation of noise in ultrasensitive signaling cascades. *Biophys. J. 82*, 2943−2950.
(46) Salis, H. M., Mirsky, E. A., and Voigt, C. A. (2009) Automated design of synthetic ribosome binding sites to control protein expression. *Nat. Biotechnol. 27*, 946−950.
(47) McGinness, K. E., Baker, T. A., and Sauer, R. T. (2006) Engineering controllable protein degradation. *Mol. Cell 22*, 701−707.
(48) Schomburg, I., Chang, A., Placzek, S., Söhngen, C., Rother, M., Lang, M., Munaretto, C., Ulas, S., Stelzer, M., Grote, A., Scheer, M., and Schomburg, D. (2013) BRENDA in 2013: Integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA. *Nucleic Acids Res. 41*, D764−D772.
(49) Golding, I., Paulsson, J., Zawilski, S. M., and Cox, E. C. (2005) Real-time kinetics of gene activity in individual bacteria. *Cell 123*, 1025−1036.
(50) Taniguchi, Y., Choi, P. J., Li, G.-W., Chen, H., Babu, M., Hearn, J., Emili, A., and Xie, X. S. (2010) Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science 329*, 533−538.
(51) Thomas, P., Straube, A. V., and Grima, R. (2012) The slow-scale linear noise approximation: an accurate, reduced stochastic description of biochemical networks under timescale separation conditions. *BMC Syst. Biol. 6*, 39-1−39-23.

(52) Puchałka, J., and Kierzek, A. M. (2004) Bridging the gap between stochastic and deterministic regimes in the kinetic simulations of the biochemical reaction networks. *Biophys. J. 86*, 1357−1372.
(53) Kubitschek, H. E., and Friske, J. A. (1986) Determination of bacterial cell volume with the Coulter Counter. *J. Bacteriol. 168*, 1466−1467.