Determinants of bistability in
induction of the *Escherichia coli* lac operon

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Abstract

We have developed a mathematical model of regulation of expression of the *Escherichia coli* lac operon, and have investigated bistability in its steady-state induction behavior in the absence of external glucose. Numerical analysis of equations describing regulation by artificial inducers revealed two natural bistability parameters that can be used to control the range of inducer concentrations over which the model exhibits bistability. By tuning these bistability parameters, we found a family of biophysically reasonable systems that are consistent with an experimentally determined bistable region for induction by thio-methylgalactoside (Ozbudak et al. Nature 427:737, 2004). The model predicts that bistability can be abolished when passive transport or permease export becomes sufficiently large; the former case is especially relevant to induction by isopropyl-β-D-thiogalactopyranoside. To model regulation by lactose, we developed similar equations in which allolactose, a metabolic intermediate in lactose metabolism and a natural inducer of lac, is the inducer. For biophysically reasonable parameter values, these equations yield no bistability in response to induction by lactose; however, systems with an unphysically small permease-dependent export effect can exhibit small amounts of bistability for limited ranges of parameter values. These results cast doubt on the relevance of bistability in the lac operon within the natural context of *E. coli*, and help shed light on the controversy among existing theoretical studies that address this issue. The results also suggest an experimental approach to address the relevance of bistability in the lac operon within the natural context of *E. coli*.
INTRODUCTION

In 1957, Novick and Weiner discovered that *Escherichia coli* can exhibit discontinuous switching in expression of the *lac* operon, with some cells expressing a large amount of β-galactosidase (β-gal), other cells expressing a small amount, and an insignificant number of cells expressing an intermediate amount [1]. Recently, this effect was further characterized using single-cell assays of fluorescence levels in a population of *E. coli* cells carrying a *lac::gfp* reporter [2]. The population exhibited a bimodal distribution, with induced cells having over 100 times the fluorescence level of uninduced cells. These observations have been attributed to the existence of two steady states, i.e., bistability, in the induction of *lac* in *E. coli*.

Recent modeling studies have emphasized the importance of determining whether bistability in expression of *lac* is relevant within a natural context [3, 4, 5, 6, 7]. This question remains open because experimental studies have focused on the response of *lac* expression to artificial inducers, such as thio-methylgalactoside (TMG) and isopropyl-β, D-thiogalactopyranoside (IPTG), rather than the natural inducer, allolactose. This difference is critical because artificial inducers (also known as gratuitous inducers) are not metabolized by the induced enzyme, whereas the natural inducer is a metabolic intermediate in lactose degradation, which is catalyzed by the induced enzyme.

Savageau [3] found important differences between induction by IPTG vs. lactose in his theoretical treatment of bistability in the *lac* operon. In Savageau’s model, because production and decay of allolactose are both proportional to the β-gal concentration, bistability is forbidden. Expression of *lac* in response to lactose was therefore predicted not to exhibit bistability. This prediction agreed with the absence of steady-state bistability in an experimental study of populations of *E. coli* cells exposed to lactose, described in the Supplementary Material of Ref. [2]—in that study, only transient bimodal distributions of green fluorescence levels among cells were observed at some glucose concentrations. It was later noted that models with operon-independent decay of lactose (e.g., due to dilution by cell growth) could exhibit bistability [7]. Several studies using such models found either a bistable or graded response to lactose, depending on parameter values or external glucose levels [5, 6, 7, 8, 9], and in agreement with the model of Savageau, a model of van Hoek & Hogeweg [7] was explicitly shown to exhibit no bistability in the absence of operon-independent decay of allolactose. However, these studies disagree in their assessment of whether bistability is present [5, 6, 9] or absent [7, 8] in expression of *lac* among *E. coli* cells in a natural
In addition to predicting whether lac induction exhibits bistability, some studies have addressed the question of whether bistability might enhance or hinder the performance of E. coli cells. Both Savageau and van Hoek & Hogeweg found that bistability increases the time required to respond to sudden increases in environmental lactose, which can be a disadvantage in competition for nutrients. These results argue against the natural relevance of bistability in lac expression.

Another important question that has not yet been addressed is whether the experimental observations of bistability in Ref. are consistent with independent biophysical data that characterize processes relevant to regulation of lac expression. Although phenomenological models were developed to reproduce the steady-state behavior and the experimentally characterized dynamics of switching between stable steady states, these models were not constrained by independent biophysical data. For example, it is unclear whether the phenomenological models are consistent with independently measured permease transport kinetics. On the other hand, studies of bistability using more detailed, biophysical models of lac induction were either only partially constrained or did not consider the response to artificial inducers.

Here we analyze bistability in an ordinary differential equation (ODE) model of lac induction. We use ODEs because we restrict our analysis to steady-state behaviors, and because the protein concentrations in fully induced cells are \( O(10^4) \) per cell (see Parameter Values section) and have negligible fluctuations. Similar equations describe induction by lactose or artificial inducers. We first use the model to gain insight into key determinants of bistability of lac expression in response to artificial inducers, and to understand how characteristics of bistability are controlled by model parameters. We then use the resulting insight to tune the parameters of the model to match the bistable behavior observed by Ozbudak et al. and to predict mechanisms by which bistability might be abolished. Finally, like previous modeling studies, we use the model to address the question of whether lac expression might be bistable in a natural context, contributing to resolution of what is now a long-standing controversy.

**MODEL**

In our model of lac induction (Fig. 1), the following set of coupled ordinary differential equations relate the internal lactose concentration \( l \), allactose concentration \( a \), and \( \beta \)-galactosidase concentration \( \beta \):
FIG. 1: Circuitry for models of \textit{lac} induction. a) Model for induction by lactose (Eqs. (1)), including the following processes: (1) proportional production of permease (LacY) and \(\beta\)-gal (LacZ); (2) permease-mediated transport of lactose; (3) dilution of intracellular species by cell growth; (4) \(\beta\)-gal catalyzed degradation of lactose, producing both the metabolic intermediate allolactose, and the ultimate products of degradation, glucose and galactose; and (5) \(\beta\)-gal catalyzed degradation of allolactose, producing glucose and galactose. b) Model for induction by artificial inducers (Eqs. (2)), including: (1) proportional production of permease (LacY) and \(\beta\)-gal (LacZ); (2) permease-mediated transport of inducer; (3) dilution of intracellular species by cell growth and (6) passive transport of inducer.

In Eqs. (1), \(\alpha\) and \(\phi\alpha\) are the rate constants for permease-dependent lactose import and export, respectively, \(K_i\) is the Michaelis constant for permease-dependent lactose transport (assumed to

\[
\dot{l} = \frac{\alpha z (l^* - \phi l)}{K_i + l + l^*} - \frac{\beta z l}{(1 + a/K_{m,a}) K_{m,l} + l} - \gamma l, \quad (1a)
\]

\[
\dot{a} = \frac{\nu \beta z l}{(1 + a/K_{m,a}) K_{m,l} + l} - \frac{\delta z a}{(1 + l/K_{m,l}) K_{m,a} + a} - \gamma a \quad \text{and} \quad (1b)
\]

\[
\dot{z} = c\gamma + \frac{e\gamma a^n}{K_z^n + a^n} - \gamma z. \quad (1c)
\]
be the same for import and export), $\beta$ and $K_{m,l}$ are the rate constant and Michaelis constant for lactose degradation, $\nu$ is the branching fraction of lactose degradation to allolactose, $\delta$ and $K_{m,a}$ are the rate constant and Michaelis constant for allolactose degradation, $\gamma$ is the rate of dilution due to cell growth, $c_\gamma$ and $\epsilon_\gamma$ are the basal and inducible rates of $\beta$-galactosidase production, $K_z$ is the allactose concentration at half-maximal induction of $\beta$-galactosidase production, and $n$ is the Hill number for lactose induction of $\beta$-galactosidase production.

The metabolic fluxes in Eqs. (1) include the effects of competition between allolactose and lactose for access to $\beta$-galactosidase ($\beta$ and $\delta$ terms). Because shuttling of galactosides across membranes occurs through a single permease channel \[11\], we also consider the influence of competition between external and internal lactose for access to permease ($\alpha$ and $\phi$ terms); however, as a simplification, we do not consider transitions among distinct internal states of the permease \[11\].

To focus on the operating conditions of the system that are most relevant to lactose utilization by \textit{E. coli}, we only consider regulation in the absence of glucose. This focus is appropriate because, in the presence of glucose, \textit{lac} is not essential for growth, and induced $\beta$-galactosidase levels are low \[12\].

Similarly, the model of artificial induction of \textit{lac} (Fig. 1b) is given by

\begin{align}
\dot{l} &= \alpha_0 \left( l^* - l \right) + \alpha_z \frac{\left( l^* - \phi l \right)}{K_i + l + l^*} - \gamma l \\
\dot{z} &= c_\gamma + \frac{\epsilon_\gamma l^n}{K^n_z + l^n} - \gamma z.
\end{align}

In Eqs. (2), variables and parameters have the same meaning as in Eqs. (1), except $l$ and $l^*$ correspond to the level of internal and external artificial inducer (e.g., IPTG or TMG), respectively, and $\alpha_0$ is the rate constant for leakage across the membrane.

In Eqs. (1) and Eqs. (2), protein expression is lumped with gene expression, and the dependence of promoter activity on the level of signal (IPTG, TMG, or allolactose) is modeled using a simple Hill function, which is significantly simpler than other models \[5, 6, 7, 8, 9, 13, 14\]. On the other hand, Eqs. (1) considers the effects of competition among substrates in permease transport and metabolic processes, unlike other models of \textit{lac} induction \[2, 3, 4, 5, 6, 7, 8, 9, 13, 15\]. Compared to the model of Savageau \[3, 4\], Eqs. (1) considers operon-independent decay of allolactose, without which bistability in response to lactose is impossible \[3, 4, 7\], as discussed above. Overall, Eqs. (1) and Eqs. (2) are less detailed than the \textit{lac} induction models used in Refs. \[13, 5, 6, 7, 8, 14\].
and [9], and are more detailed than those used in Refs. [3], [4], [15], and [2], and they therefore constitute intermediate complexity equations describing $lac$ induction. Compared to the simpler models, the intermediate level of detail provides increased contact between model parameters and biophysical measurements, and compared to more detailed models, it facilitates analysis of the equations and interpretation of the results.

**PARAMETER VALUES**

We used the parameter values and ranges listed in Table I to analyze bistability in Eqs. (1) and Eqs. (2). The values in the table were obtained as follows:

- $\gamma$. We assume the generation time under the conditions in Ref. [2] is 30-60 min. We note, however, that this time might be very different for *E. coli* growing under stress in the gut; this represents a source of uncertainty concerning the biological relevance of our predictions.

- $\alpha_0$. We assume that $\alpha_0 = 0$ except for the case of IPTG, where we explore a range consistent with that considered in Ref. [8].

- $\alpha$. An approximate range of 1-100 s$^{-1}$ for sugar transport turnover numbers was obtained from the review by Wright et al. [16]. The range is broader than measured values [17] because measurements were made at 25°C rather than at the physiological temperature of 37°C in the host environment of the gut that we are focusing on here, and at which measurements in Ref. [2] were performed. The nominal value of 1000 min$^{-1}$ was estimated from Ref. [17] assuming the production rate of permease is the same as that of functional $\beta$-gal. Because permease is a monomer while $\beta$-gal is a tetramer, this assumption entails a four-fold smaller production rate for permease. This seems possible, as (1) galactoside acetyltransferase (GATase) monomer synthesis is eight-fold smaller than $\beta$-gal monomer synthesis; (2) due to incomplete operon transcription and the order of genes in the operon ($lacZYA$), the amount of mRNA transcribed from the GATase gene ($lacA$) and permease gene ($lacY$) is smaller than that from the $\beta$-gal gene ($lacZ$); (3) there is some evidence that permease is made in smaller amounts than $\beta$-gal [18].

- $\phi$. We assume no export flux through permease in the artificial induction model, and then examine the consequences of introducing such a flux on bistability. Guided by Ref. [11], for
the lactose model, we use a nominal efflux rate constant ($\phi_\alpha$) of half the value of the influx rate constant $\alpha$, and allow the value to decrease in the search for bistable conditions.

- $K_i$. For simplicity, we assume the same Michaelis constant for permease import and export–a nominal value of 0.5 mM was obtained from Ref. [17]. The range was applied as per $\alpha$, and encompasses measured values [17, 19, 20].

- $\beta$. A total lactose turnover number for $\beta$-galactosidase of $2.85 \times 10^4 \text{ min}^{-1}$ is estimated from a measured value of $V_{max} = 61.3 \mu \text{mol min}^{-1} \text{ mg}^{-1}$ in Ref. [21]. This estimate is an order of magnitude greater than the value $3.6 \times 10^3 \text{ min}^{-1}$ given in Ref. [22], but the two estimates agree closely when one considers that $\beta$-gal converts about half of its lactose substrate to glucose and galactose, rather than allolactose, and that the enzyme is composed of four monomeric catalytic subunits. The estimate given in Ref. [22] is appropriate for total turnover of lactose on a per monomer basis. Like for $\alpha$, because measurements were performed at 30°C, we consider a range of values ten times lower to ten times higher than the nominal value.

- $K_{m,l}$. The nominal value was obtained directly from Ref. [23]. As for $\beta$, because of temperature considerations, we use a range from ten times lower to ten times higher than the nominal value.

- $\nu$. The value $\nu = 0.468$ was calculated from the total rate of $\beta$-gal degradation of lactose and the partial flux from lactose to allolactose reported in Ref. [21]. We take it to be a constant because the ratio of reaction products was found to be insensitive to temperature changes between 30°C and 0°C.

- $\delta$. An allolactose turnover number for $\beta$-gal of $2.3 \times 10^4 \text{ min}^{-1}$ is estimated from a measured value of $V_{max} = 49.6 \mu \text{ mol min}^{-1} \text{ mg}^{-1}$ in Ref. [23]. As for $\beta$, because of temperature considerations, we use a range from ten times lower to ten times higher than the nominal value.

- $K_{m,a}$. The nominal value was obtained directly from Ref. [23]. As for $\beta$, because of temperature considerations, we use a range from ten times lower to ten times higher than the nominal value.
• \( \epsilon \). Using a production rate of 5 \( \beta \)-gal tetramers per cell per second for a 48 min generation time [24], 14,400 molecules are produced during a generation at full induction—this is the number of molecules in the cell after doubling (supporting our choice of a noiseless model). Assuming a 1 \( \mu \text{m}^3 \) mean cell volume [25] and linear volume increase in time [26], the volume after doubling is approximately 0.7 \( \mu \text{m}^3 \), leading to a concentration of 34,286 nM.

• \( c \). This value is derived from \( \epsilon \), assuming a 1000-fold increase in \( \beta \)-galactosidase levels upon induction [27].

• \( K_z \) and \( n \). These values are estimated from IPTG induction data in permease knockout cells both from Ref. [28], Fig. 15 and from data compiled in Ref. [29], Figs. 1 and 2. The nominal value \( n = 2 \) was estimated from the slopes of the curves in the figures, and \( K_z \) was determined by estimating from the figures the concentration of IPTG at half-maximal induction. The nominal value of \( 10^5 \) nM was estimated from data compiled in Ref. [29]. To determine the range, an approximate lower value of \( 10^4 \) nM was obtained from Ref. [28], and we allowed for an upper value of \( 10^6 \) nM to account for potential differences between induction by IPTG and TMG or lactose.

RESULTS

We first used Eqs. (2) to determine how parameter values control bistability in the steady-state response of \( lac \) expression to artificial inducers. To detect and characterize bistability for a given set of parameter values, we solved for \( z(l) \) and \( l^*(l) \) as rational functions of \( l \). Bistability in \( lac \) expression exists when the line describing steady-state levels of \( z \) vs. \( l^* \) adopts a characteristic “S” shape, as shown in Fig. 2. Within the bistable range of \( l^* \), the highest and lowest levels of \( z \) are stable steady-state solutions and the intermediate level of \( z \) is an unstable steady-state solution of Eqs. (1). The bistable range is defined by the lower \( (l^* = L) \) and upper \( (l^* = U) \) turning points, as illustrated in Fig. 2. An analogous signature of bistability can be seen in examining steady-state levels of \( l \) vs. \( l^* \) (not shown). For a model with given parameter values, \( L \) and \( U \) can be located by finding the roots of either \( dl^*/dz \) or \( dl^*/dl \) using an eigenvalue solver.

We analyzed Eqs. (2) for systems with sets of parameter values drawn from the ranges in Table I, taking \( \alpha_0 = 0 \), \( \phi = 0 \), and \( n = 2 \). Sets of 100 values each for \( K_i \) and \( K_z \) were obtained using
### TABLE I: Parameter values.

| Param  | Description                                      | Nominal      | Range                                  |
|--------|--------------------------------------------------|--------------|----------------------------------------|
| $\gamma$ | growth rate                                      | –            | 0.0116 min$^{-1}$ – 0.0231 min$^{-1}$   |
| $\alpha_0$ | passive transport rate constant                  | 0            | 0 – 1.35 min$^{-1}$                    |
| $\alpha$ | permease import turnover number                  | 600 min$^{-1}$ | 6 $\times$ 10$^4$ min$^{-1}$ – 6 $\times$ 10$^6$ min$^{-1}$ |
| $\phi$  | ratio of permease export to import turnover numbers | 0 (artificial inducers) or 0.5 (lactose) | 0 – 0.5                                 |
| $K_i$  | permease Michaelis constant                      | 5 $\times$ 10$^9$ nM | 5 $\times$ 10$^4$ nM – 5 $\times$ 10$^6$ nM |
| $\beta$ | $\beta$-gal lactose turnover number              | 2.85 $\times$ 10$^4$ min$^{-1}$ | 2.85 $\times$ 10$^3$ min$^{-1}$ – 2.85 $\times$ 10$^5$ min$^{-1}$ |
| $\nu$  | lactose $\rightarrow$ allolactose $\beta$-gal branching fraction | 0.468        | –                                      |
| $K_{m,l}$ | $\beta$-gal lactose Michaelis constant          | 2.53 mM      | 0.253 mM – 25.3 mM                     |
| $\delta$ | $\beta$-gal allolactose turnover number         | 2.30 $\times$ 10$^4$ min$^{-1}$ | 2.30 $\times$ 10$^3$ min$^{-1}$ – 2.30 $\times$ 10$^5$ min$^{-1}$ |
| $K_{m,a}$ | $\beta$-gal allolactose Michaelis constant      | 1.2 mM       | 0.12 mM – 12.0 mM                      |
| $\epsilon$ | fully induced $\beta$-gal level                 | 34285 nM     | –                                      |
| $c$    | basal $\beta$-gal level                          | 34.3 nM      | –                                      |
| $K_z$  | signal level at half-maximal lac induction       | 10$^9$ nM    | 10$^4$ nM – 10$^6$ nM                 |
| $n$    | Hill number for signal-dependent lac induction   | 2            | –                                      |

logarithmically even sampling over their allowed ranges. Because the steady-state solutions of Eqs. (2) only depend on $\alpha$ and $\gamma$ through the ratio $\alpha/\gamma$, rather than sampling $\alpha$ and $\gamma$ individually, we obtained 100 values of $\alpha/\gamma$ using logarithmically even sampling between the upper and lower bound computed from Table I. This sampling scheme yielded 100 $\times$ 100 $\times$ 100 = 10$^6$ systems with different values of $(\alpha/\gamma, K_i, K_z)$.

We found that all 10$^6$ systems exhibited some degree of bistability in response to induction by artificial inducers. The dependence of the range of bistability on model parameters was further analyzed using two measures that we introduce here: the ratio $U/L$, and the product $UL$. We used
FIG. 2: An example of a system from Eqs. (2) with the upper ($U$) and lower ($L$) turning points consistent with the results in [2]. The parameter values are $\gamma = 0.231 \text{ min}^{-1}$, $\alpha = 60 \text{ min}^{-1}$, $K_z = 123,285 \text{ nM}$ and $K_i = 1,077,217 \text{ nM}$.

these measures to estimate the percentage of systems for which bistability might be observable in an experiment like that in Ref. [2]. By inspecting the measurement errors in Ref. [2], we estimate that systems with $U/L > 1.1$ and $UL > 0.01 \mu M^2$ exhibit bistability that is favorable for experimental observation (i.e., difficult to detect), and that systems with either $U/L < 1.1$ or $UL < 0.01 \mu M^2$ exhibit bistability that is unfavorable for experimental observation. Among systems with parameter values sampled as described above, by these criteria, we predict that experimental observation of bistability would be favorable for 65% of systems, and unfavorable for 35% of systems.

Increasing either $\alpha_0$ or $\phi$ above zero tends to reduce or abolish bistability in artificially induced systems. As $\alpha_0$ is increased (Fig. 3), first $U$ begins shifting to lower values of $l^*$, then $L$ begins shifting to higher values of $l^*$, leading to an asymptotic behavior in which bistability is abolished. Like changes in $\alpha_0$, as $\phi$ is increased (Fig. 4), $L$ shifts to higher values of $l^*$; however, by contrast, $U$ does not initially show a significant change. As $\phi$ is increased further, the entire induction curve begins to shift to higher levels of $l^*$.

To compare Eqs. (2) to the data in Ref. [2], we first selected a subset of systems for which
FIG. 3: Effects of variations in the $\alpha_0 > 0$ parameter on an artificially induced system with $\phi = 0 \, \text{min}^{-1}$ and $\alpha_0 = 10^{-k} \, \text{min}^{-1}, k = 0, \ldots, 4$. The other parameters are given by $n = 2$, $\gamma = .0231 \, \text{min}^{-1}$, $\epsilon = 34286 \, \text{nM}$, $c = 34.3 \, \text{nM}$, $K_i = 5 \times 10^6 \, \text{nM}$, $K_z = 10^4 \, \text{nM}$ and $\alpha = 60 \, \text{min}^{-1}$.

The bistable region is in the same neighborhood as that in Ref. [2]: from $3 \, \mu\text{M}$ to $30 \, \mu\text{M}$ TMG. Considering this range, out of the $10^6$ systems sampled, we selected 187,108 systems for which $L > 1 \, \mu\text{M}$ and $U < 100 \, \mu\text{M}$ for further analysis. Interestingly, we found that all of these systems collapse to a single curve when displayed in the space of $\log_{10}(U/L)$ vs. $\log(K_i/K_z)$ (Fig. 5), indicating that $U/L$ can be precisely tuned using the parameter $X = K_i/K_z$. As shown in Fig. 5, the dependence was accurately modeled using the equation

$$\log_{10}(U/L) \approx \frac{(K_i/K_z)^{.93}}{(K_i/K_z)^{.93} + (.27)^{.93}} - \frac{1}{10} \geq 0. \quad (3)$$

Next, we found that, at a given value of $X = K_i/K_z$, without changing the value of $U/L$, $UL$ could be tuned precisely using the parameter $Y = K_iK_z\gamma/\alpha$. As shown in Fig. 6, this dependence was accurately modeled using the equation

$$\log_{10}(UL) = C_0(X) + C_1(X) \log_{10}(Y). \quad (4)$$

Figure 7 shows the $X$-dependence of the parameters $C_0(X)$ and $C_1(X)$, obtained numerically.
FIG. 4: Effects of variations in the $\phi > 0$ parameter on an artificially induced system with $\alpha_0 = 10^{-4} \text{ min}^{-1}$ and $\phi = 0$ and $10^{-k} \text{ min}^{-1}$, $k = 1, \ldots, 4$. All of the scales are in $\mu \text{M}$. The other parameter values are as in Figure 3.

using systems with similar values of $X$. For the range of systems considered here, we found that $C_0(X)$ could be fit using a third order polynomial in $\log_{10}(X)$, and that $C_1(X)$ could be taken as a constant.

The above phenomenological results provide a prescription for tuning the range of bistability exhibited by an artificially induced system. First, the value of $U/L$ can be specified by choosing a value of the parameter $X = K_i/K_z$ using Eq. (3). Then, using this value of $X$, the value of $UL$ can be specified by choosing a value of the parameter $Y = K_z K_i \alpha/\gamma$ using Eq. (4) and the empirically determined $C_0(X)$ and $C_1(X)$ (Fig. 7). We used this prescription to obtain a family of systems that are consistent with the parameter values in Table I and that exhibit a range of bistability consistent with that observed in Ref. [2], with $\log_{10}(U/L) \approx 0.86$ and $\log_{10}(UL) \approx 1.92$. An example of the steady-state behavior of one such system is illustrated in Figure 4.

We used similar methods to analyze Eqs. (1) which describe induction by lactose. No bistability was present in the system with nominal parameter values from Table I (Fig. 8) with $\phi = 0.5$, which
FIG. 5: Modeling the width as a sigmoid function of $K_i/K_z$. Only a sample of data points are shown.

is consistent with the theory of Savageau [3] and the Supplementary Material of Ref. [2]. However, guided by the results for artificial inducers in Fig. 4, we examined systems with $\phi = 0$. Although the system with nominal parameter values and $\phi = 0$ did not exhibit bistability, other systems that have parameter values consistent with the ranges in Table I did exhibit bistability. We then located the system that exhibits the largest values of $U/L$ and $UL$; for this case, $\alpha$, $\beta$, $\delta$ and $K_z$ assume their lowest values in Table I while $\gamma$, $K_{m,I}$, $K_{m,a}$ and $K_i$ assume their highest values (Fig. 9). The curve in Fig. 9 illustrating bistability characteristics for this system closely resembles a similar curve shown in van Hoek & Hogeweg [7], Fig. 2B. Thus, although our model is less detailed than theirs, it can exhibit comparable steady-state behavior.

To estimate the distribution of systems exhibiting the different qualitative behaviors, as for the case of artificial inducers, we analyzed $10^4$ systems with randomly sampled parameter values, all with $\phi = 0$. We predict 99.87% of these systems to exhibit no bistability, 0.05% to exhibit bistability favorable for observation ($U/L > 1.1$ and $UL > 0.01 \mu M^2$), and 0.08% to exhibit bistability that is unfavorable for observation ($U/L < 1.1$ or $UL < 0.01 \mu M^2$). However, as observed for Eqs. (2), increasing $\phi$ to even a small fraction of its nominal value rapidly abolishes bistability for all combinations of other parameter values in Eqs. (1) (Fig. 10).
FIG. 6: Using the $C_0$ and $C_1$ in Figure 7 results in a linear relation between the center $\log_{10}(UL)$ and $\log_{10}(K_iK_z\gamma/\alpha)$.

CONCLUSIONS

For the equations describing induction by artificial inducers, we found that the range of external inducer concentrations over which systems exhibit bistability is precisely controllable by two rational combinations of model parameters. By adjusting these parameters, we were able to demonstrate agreement with the bistable range for TMG induction from Ref. [2]. However, in achieving this agreement, we assumed that permease-dependent efflux of artificial inducers is negligible ($\phi = 0$). We have not found independent biophysical data to constrain this parameter for artificial inducers, and therefore predict that it has a value much less than the value of roughly 0.5 that has been measured for lactose.

To achieve agreement with the bistable range of roughly 3 $\mu$M to 30 $\mu$M in Ref. [2], $c$ and $\epsilon$ in Eqs. (2) were tuned to exhibit a 1000-fold induction of protein expression. While this value is reasonable based on previous studies, it does disagree with the roughly 100-fold induction of GFP expression reported in Ref. [2]. We did analyze systems with alternative values of $c$ and $\epsilon$ that yield 100-fold induction; however, none of them exhibited bistable ranges that agree with
Further studies will be required to understand why Eqs. (2) does not simultaneously agree with both the bistable range and maximal induction of the experimental $lac :: gfp$ reporter system. In addition to model refinement, it would be fruitful to seek systematic differences between expression from chromosomal $lac$ and the plasmid-based $lac :: gfp$ reporter system used in Ref. [2].

The lack of bistability observed for induction by lactose agrees with modeling studies concluding that bistability in $lac$ expression is irrelevant to $E. coli$ in a natural context [3, 4, 7, 8]. Thus, although bistable behavior in $lac$ is now well-documented [1, 2, 30], because it has only been experimentally observed using artificial inducers, its relevance within the natural context of $E. coli$ is doubtful. Indeed, it is surprising that the $lac$ operon has been considered to be a paradigm of bistability in gene regulation, considering the gaps in understanding that remain after so many careful experimental and theoretical studies.

The present results predict that bistable behavior can be promoted by (1) hindering the kinetics of permease transport ($\alpha, K_i$) and $\beta$-gal catalysis ($\beta, \delta, K_m,t, K_m,a$); (2) lowering the required level of allolactose for half-maximal $lac$ expression ($K_z$); and (3) accelerating cell growth ($\gamma$). These predictions suggest genetic targets for engineering $E. coli$ strains that exhibit a clear signature of bistability.
bistability. Experiments to compare the behavior of such strains with wild-type cells would help to clarify whether bistability in lac expression is relevant in a natural context.
FIG. 9: Bistability in the $\phi = 0$ lactose-induced system with $\alpha$, $\beta$, $\delta$ and $K_z$ at their lowest values in Table II and $\gamma$, $K_{m,l}$, $K_{m,a}$ and $K_i$ at their highest values. This is the system that exhibits the largest values of $\frac{U}{L}$ and $UL$ within the allowed ranges of parameter values.

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FIG. 10: This is the same system as in Figure 9 with $\phi = 0$ and $10^{-k} \text{ min}^{-1}$, $k = 1, \ldots, 4$.

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