Switching off: the phenotypic transition to the uninduced state of the lactose uptake pathway

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The lactose uptake-pathway of E. coli is a paradigmatic example of multistability in gene-regulatory circuits. In the induced state of the lac-pathway, the genes comprising the lac-operon are transcribed, leading to the production of proteins which import and metabolize lactose. In the uninduced state, a stable repressor-DNA loop frequently blocks the transcription of the lac-genes. Transitions from one phenotypic state to the other are driven by fluctuations, which arise from the random timing of the binding of ligands and proteins. This stochasticity affects transcription and translation, and ultimately molecular copy numbers. Our aim is to understand the transition from the induced to the uninduced state of the lac-operon. We use a detailed computational model to show that repressor-operator binding/unbinding, fluctuations in the total number of repressors, and inducer-repressor binding/unbinding all play a role in this transition. Based on the timescales on which these processes operate, we construct a minimal model of the transition to the uninduced state and compare the results with simulations and experimental observations. The induced state turns out to be very stable, with a transition rate to the uninduced state lower than $2 \times 10^{-9}$ per minute. In contrast to the transition to the induced state, the transition to the uninduced state is well described in terms of a 2D diffusive system crossing a barrier, with the diffusion rates emerging from a model of repressor unbinding.

Introduction

Multistable gene regulatory circuits play an important role in diverse biological processes like embryo development [1] [2], viral reproduction [3] [4], and nutrient uptake in bacteria [5] [6]. Despite the term “multistable”, regulatory circuits are never truly multistable, as transitions from one phenotypic state to another occur due to random fluctuations: The processes involved in gene expression regulation (changes in operator state, transcription, and translation) are intrinsically stochastic, as is ligand binding. Another source of stochasticity affecting molecular copy numbers is cell division, leading to the random partitioning of molecules in the two daughter cells. These fluctuations can take the system from one (long-lived) phenotypic state to another. The particular fluctuations driving a phenotypic transition vary from system to system. For instance, the transition to competence in B. subtilis is driven by fluctuations in the numbers of the ComK protein [7], while in the arabinose uptake pathway it is entirely driven by the initial distribution of pump proteins in the cell [5].

In this paper we focus on the lactose uptake pathway (lac-pathway), which has been studied extensively since the 1960s [6] [8] [16]. Following early discoveries by Jacob and Monod [8], and Novick and Weiner [9], the lac-pathway has become a paradigmatic system for study of gene regulation [13] [14]. The last decade has seen renewed interest in the lac-pathway due to its multistability, that is the ability of the pathway to sustain different concentrations of a particular protein (or sets of proteins) for long times [6] [10] [12] [15] [16]. The induced and uninduced states define two distinct phenotypes differing in their lactose uptake. The induced state persists due to feedback; the more lactose is present in the cell, the more lac-repressors have a reduced affinity for the lac regulatory region and the more pumps are produced and import further lactose. Nevertheless, the number of pumps fluctuates stochastically and will (with a small probability per unit time) cross a threshold to the uninduced state.

In previous work, we have combined a detailed mechanistic model of the lac-pathway with flow-cytometry experiments to understand the transition from the uninduced to the induced state [15]. We found that this transition occurs when the lac-repressor unbinds from the lac regulatory region and remains unbound for a period of time sufficient to import enough lactose to deactivate all repressors. A simple model of this process gives a transition rate to the induced state which decays exponentially with the inverse of the repressor concentration. This result is borne out experimentally very precisely and over several orders of magnitude (see Fig. 5 of [15]).

However, the reverse transition (from the induced to the uninduced state) is still poorly understood. Our goal here is to identify the sources of stochasticity which affect the transition to the uninduced state and to set up a model of the lac-pathway which incorporates only the stochastic elements relevant to this transition. With this approach, we aim to bridge the gap between simple models of gene regulatory networks, which are tractable using the tools of stochastic processes and statis-
Materials and methods

The lac pathway is formed by the lac genes lacY, lacZ, and lacA, which are under joint regulatory control, thus forming a so-called operon. In the induced state of the lac-pathway, lactose (‘inducer’) is imported across the cell membrane by the LacY protein (‘pumps’) and metabolized by the enzyme LacZ into glucose and galactose. Allolactose, a lactose variant originating from LacZ activity, binds to the repressor of the lac genes and drastically reduces the affinity of the lac-repressor to its DNA binding sites [6, 14]. The lac repressor is formed by a dimer of LacI dimers which are expressed constitutively. The reduced affinity causes the repressor to unbind from the lac regulatory region, enabling the transcription of the lac genes and production of the LacY proteins (‘pumps’) and further import of lactose. In the uninduced state on the other hand, LacI frequently binds to two DNA sites in the regulatory region of the lac-operon and forms a DNA-repressor loop that effectively blocks transcription of the lac-genes and thus the import of lactose into the cell. For a graphical representation, see Fig. 1.

A detailed mechanistic model of the lac-pathway. Our model describes the transcription and translation of mRNA and protein, both of LacY proteins (lactose importer or ‘pumps’) and of LacI proteins (repressors), repressor binding to DNA at its binding sites, DNA looping, the uptake of lactose (inducer) or its analog into a cell, and the passive diffusion of inducers into the cell. For details, see SI Section 1.
Thus, several processes taking place on different timescales provide the smoothing procedure to all constituents of the mechanistic model using the Gillespie algorithm [31]. To measure the mean first passage time (MFPT) from the induced to the uninduced state at a given external inducer concentration, previously induced cells (approximately $10^4$ pump proteins at time $t = 0$) are simulated with that inducer concentration until the pump protein number crosses a target corresponding to the uninduced state ($O(100)$).

To pinpoint the relevant fluctuations affecting this transition, we used a smoothing procedure introduced in [15]. This procedure reduces the amplitude of fluctuations of a particular component in the pathway, allowing the identification of those fluctuations which influence the rate of transitions to the uninduced state. The smoothing is based on decreasing the step size of a particular reaction, while simultaneously increasing its rate by the same factor ('one tenth of a molecule produced at ten times the rate'). This leaves the mean number of a particular molecule unaffected, but reduces the variance, see [15].

**Experimental determination of transition rates to the uninduced state.** We used the *E. coli* strain CH458 which has a green fluorescent protein (GFP) gene cassette inserted just after the lac-genes. Since the GFP gene is co-located with the lac-genes, the number of GFP and lac-proteins in the cell are correlated. High levels of fluorescence indicate cells in the induced state, while uninduced cells have low levels of fluorescence.

We used TMG, a non-metabolizable analog of lactose as an inducer. We exposed populations of previously induced cells to different concentrations of the inducer and used flow cytometry to determine the fraction of cells that had switched to the uninduced state at a particular later time. Fitted to an exponential decay, measurements at different times yield the transition rate from the induced to the uninduced state at a given concentration of inducer (see SI Section 2 and [15] for details).

**Results**

In numerical simulations of our mechanistic model, we applied the smoothing procedure to all constituents of the lac-pathway and their binding states in turn. We found that repressor-operator binding/unbinding, inducer-repressor binding/unbinding, and fluctuations in the total number of repressors all affect the rate of transitions to the uninduced state. Thus, several processes taking place on different timescales are involved in the transition. On the other hand, fluctuations in pump numbers due to translation bursts do not significantly affect the transition rate.

These results are compatible with the following picture of the transition to the uninduced state: The transition to the uninduced state occurs when the copy number of pumps becomes sufficiently low. This happens as the result of a series of prolonged periods when the lac-genes are transcriptionally inactive, interspersed with shortened periods when the lac-genes are transcribed [17, 18, 22]. Fluctuations to high numbers of repressors lead to fast repressor binding and shorten the transcriptionally active periods. The length of inactive periods, on the other hand, is determined by the rate of repressor unbinding. Repressor unbinding is itself a multi-step process, since the repressor can bind the regulatory region at two sites simultaneously, and its affinity to these sites changes with the number of inducers bound to the repressor. Fluctuations in the number of inducers bound to a repressor thus affect how long it takes for this repressor to unbind from the regulatory region.

Based on this picture, we propose a minimal quantitative model of the transition to the uninduced state: On timescales which are long compared to the the time intervals between binding/unbinding of repressors, but short compared to the mean first passage time, the number of pumps is described as a diffusive process [17]. Drift and diffusion of this process are affected by the rate of repressor binding and repressor unbinding. In the following sections, we discuss this diffusion approximation for the pump copy number, calculate the repressor unbinding rate and how it depends on the inducer concentration, describe the fluctuations in the number of repressors in terms of a second diffusive process. Putting these elements together leads to a Fokker–Planck equation in two variables, which describes the stochastic dynamics of pump and repressor numbers. We calculate the mean-first passage time from the induced state to the uninduced state under this Fokker–Planck equation and compare the results to simulations of the full mechanistic model as well as single-cell experiments.

**Production of pump proteins.** We start with a master equation for protein production with repressors binding and unbinding from the operator at rates $\beta$ and $\eta$ respectively [17]. This is a standard model of gene regulation; later we will add the dependencies of $\beta$ and $\eta$ on repressor numbers and inducer numbers which are specific to the lac pathway, $p_0 \beta (t)$ denotes the probability that the lac-genes are transcriptionally inactive (the operator is bound by a repressor, a state denoted 0), and there are $Y$ pump proteins in the cell, while $p_1 \beta (t)$ denotes the probability that the lac-genes are transcriptionally active (the operator is free of a repressor; state 1) and there are $Y$ pump proteins in the cell. The production rates of the pumps when repressor is bound and unbound from operator are denoted by $\xi$ and $\xi$, respectively (see SI Table. I), while the rate at which the pump number is reduced through degradation and cell division is denoted by $\varphi$. The master equation of this process
Writing the Taylor series of a function as a system of equations, we follow the standard route outlined in [17]. To derive a Fokker–Planck equation corresponding to this system of equations, we first compute the drift and diffusion terms from our calculations, Eq. (4) (without any parameter fitting). We observe a very good match between the detailed mechanistic model and the results of Fig. 2. The points of zero drift, $Y^{\text{OFF}}$ and $Y^{\text{ON}}$, represent the stable points of the induced and uninduced states respectively, while $Y^C$ is the separatrix between them. In the panel below, we plot the diffusion from simulations of our detailed mechanistic model along with the theoretical diffusion term from Eq. (5). The agreement is good at intermediate and high pump copy numbers, while at low pump copy numbers some discrepancy arises.

Unfortunately, not all rates of repressor binding and unbinding in the presence of different numbers of inducers have been measured experimentally (to the best of our knowledge). We use the detailed balance condition to infer some of these rates, see SI Section 1.1 for details.

To compute the effective rate $\eta$ of the unbinding of a repressor-DNA loop (see Fig. 3), we first compute the dissociation rate $\langle \eta_j \rangle$ of a repressor with a constant number $j$ of inducers bound to it (SI Section 4). Then we introduce effective rates at which inducers bind to and unbind from that repressor, see Fig. 5. The network of stochastic transitions in Fig. 5 yields a set of linear equations for the residence times $\langle \tau_j \rangle$ of a repressor on DNA, given that the repressor initially has $j$ inducers bound to it, see SI Section 4 for details. These equations are
FIG. 3: **Repressor unbinding.** The repressor unbinding rate depends on the number of inducers bound to the repressor, which changes over time. From left to right, 0, 1, 2, 3 or 4 inducers are bound to the repressor, with the changes in the number of inducers indicated by horizontal arrows. The effective rates of inducer binding to the inducer-DNA system are given by \( \hat{b}_j \) and the dissociation rate for a single inducer is given by \( \hat{d} \) (see SI Table I and SI Section 4.2 for details). The two legs of the repressor can unbind individually (diagonal arrows in each subplot). When both legs have unbound, the repressor dissociates from the regulatory region. The “exit rate” at which this happens, starting from a given initial number of inducers bound to the repressor, is calculated in SI Section 4.

\[
\begin{align*}
\tau_0 &= \frac{\eta_0}{(\eta_0 + \hat{b}_0 I)^2} - \frac{\hat{b}_0 I}{\eta_0 + \hat{b}_0 I} \left( \frac{1}{\eta_0 + \hat{b}_0 I} + \tau_1 \right) = 0 \\
\tau_1 &= \frac{\eta_1}{(\eta_1 + \hat{d} + \frac{3}{4} \hat{b}_1 I)^2} - \frac{\hat{d}}{\eta_1 + \hat{d} + \frac{3}{4} \hat{b}_1 I} \left( \frac{1}{\eta_1 + \hat{d} + \frac{3}{4} \hat{b}_1 I} + \tau_0 \right) - \frac{\frac{3}{4} \hat{b}_1 I}{\eta_1 + \hat{d} + \frac{3}{4} \hat{b}_1 I} \left( \frac{1}{\eta_1 + \hat{d} + \frac{3}{4} \hat{b}_1 I} + \tau_2 \right) = 0 \\
\tau_2 &= \frac{\eta_2}{(\eta_2 + 2\hat{d} + \frac{1}{2} \hat{b}_2 I)^2} - \frac{2\hat{d}}{\eta_2 + 2\hat{d} + \frac{1}{2} \hat{b}_2 I} \left( \frac{1}{\eta_2 + 2\hat{d} + \frac{1}{2} \hat{b}_2 I} + \tau_1 \right) - \frac{\frac{1}{2} \hat{b}_2 I}{\eta_2 + 2\hat{d} + \frac{1}{2} \hat{b}_2 I} \left( \frac{1}{\eta_2 + 2\hat{d} + \frac{1}{2} \hat{b}_2 I} + \tau_3 \right) = 0 \\
\tau_3 &= \frac{\eta_3}{(\eta_3 + 3\hat{d} + \frac{1}{4} \hat{b}_3 I)^2} - \frac{3\hat{d}}{\eta_3 + 3\hat{d} + \frac{1}{4} \hat{b}_3 I} \left( \frac{1}{\eta_3 + 3\hat{d} + \frac{1}{4} \hat{b}_3 I} + \tau_2 \right) - \frac{\frac{1}{4} \hat{b}_3 I}{\eta_3 + 3\hat{d} + \frac{1}{4} \hat{b}_3 I} \left( \frac{1}{\eta_3 + 3\hat{d} + \frac{1}{4} \hat{b}_3 I} + \tau_4 \right) = 0 \\
\tau_4 &= \frac{\eta_4}{(\eta_4 + 4\hat{d})^2} - \frac{4\hat{d}}{\eta_4 + 4\hat{d}} \left( \frac{1}{\eta_4 + 4\hat{d}} + \tau_3 \right) = 0,
\end{align*}
\]

where \( \hat{b}_j \) are the effective rates of inducer binding to the inducer-DNA system and \( \hat{d} \) is the dissociation rate for a single inducer.

We compute the effective residence time of a repressor on DNA \( \frac{1}{\eta} \) by averaging the \( \{ \tau_j \} \) obtained by solving equations (6–10), over the probabilities that a repressor-DNA loop has \( j \) inducers bound to it. These probabilities \( \{ \hat{r}_j \} \) of a DNA-repressor loop being formed with \( j \) bound inducers can be calculated given the relative number of repressors and inducers in the cell and the fact that inducer binding and unbinding
is fast compared to the timescale over which the number of repressors change (see SI Section 4.4). The effective unbinding rate $\eta$ is then given by

$$\eta = \left( \sum_{j=0}^{4} \hat{r}_j \tau_j \right)^{-1}.$$  
(11)

In Eqn. (11), the residence times $\{\tau_j\}$ and the probabilities $\{\hat{r}_j\}$ are functions of the number of inducers in the cell, which in turn is a function of number of pump proteins and external inducer concentration. At a fixed number of pumps, an equilibrium between inducer import and inducer dilution through cell growth is established. The mean number of inducers $I$ can then be written as a function of pump number $Y$ (see SI Table. I)

$$I(Y) = \frac{m}{\varphi E_h + E} Y,$$  
(12)

where $\varphi$ is the dilution rate, $m$ is the rate of inducer import per pump, $E_h$ is the Michaelis constant which we use as a fitting parameter, and $E$ is external TMG concentration. To the best of our knowledge, measurements of the quantities $m$ and $E_h$ are not available for TMG, the lactose analog used in our experiments [15]. From equation (12) it is clear that assumptions about the value of $m$ will strongly affect the fitting value for $E_h$. In our mechanistic model, we use the value of $m$ reported in [32] by Smirnova et. al for the sugar NPG (1260/min). We determine the Michaelis constant $E_h$ by fitting simulations of the detailed mechanistic model to experimental data on the switching from the uninduced to the induced state from [15] (the reverse transition to the one considered here, see SI Section 3 for details). The best fit was obtained for $E_h = 1.05 \times 10^7 \mu M$. For comparison, values of the Michaelis constants $E_h$ for lactulose transport by LacY ($2.4 \times 10^7 \mu M$), and sucrose ($6.7 \times 10^4 \mu M$), fructose ($3.5 \times 10^4 \mu M$) transport by CscB are reported by Sugihara et. al in [33]. Since there are significant variations between different sugars, the fitted value of $E_h$ for TMG is not implausible, however it is sensitive to other parameters of the model for which TMG-specific measurements are not available. Specifically, for large values of $E_h$, equation (12) depends on $m$ and $E_h$ only through their ratio. The value obtained for $E_h$ might thus reflect simply a value of the parameter $m$ that is not correct for the inducer TMG used here. However, while $E_h \gg 100 \mu M$ the ratio of $m$ and $E_h$ will be independent of inaccuracies in the value of $m$.

Equations (11) and (12) jointly establish a relationship between the number of pumps and the repressor dissociation rate. This is an effective rate, which results from inducers repeatedly binding/unbinding from repressors and influencing the residence time of repressors on the regulatory region. Equations (11) and (12) quantify the amount of feedback in the lac-pathway: The more pumps there are, the faster the lac-repressor will unbind from the regulatory region, enabling the production of further pumps. In Fig. 4 we compare the results from Eqs. (11) and (12) for the effective dissociation rate to the rate at which repressors unbind from the regulatory region in simulations of the detailed mechanistic model and find very good agreement. This effective repressor dissociation rate will be used below as the rate at which the lac operon turns from the transcriptionally inactive to the active state, which enters the diffusion model of the pump numbers.

**Repressor binding.** The second quantity entering the diffusion model of the pump numbers is the rate $\beta$ at which the regulatory region is bound by a repressor. This rate is proportional to the number of repressors in the cell, the rate $g$ at which repressors can find their binding site on DNA (see SI Table I for details) and the rate at which these repressors can form a repressor-DNA loop, which in turn depends on the numbers of inducers bound to the repressor. The binding rate $\beta$ is a function of the inducer numbers inside the cell via the fractions of free repressors in the cell with $j$ inducers bound to them $\{r_j\}$

$$\beta = g R \sum_{j=0}^{4} r_j \frac{c}{c + w_{4-j}},$$  
(13)

where $R$ denotes the number of repressors in the cell, $c$ is the rate at which a repressor with one leg bound to DNA forms the repressor-DNA loop. $w_{4-j}$ denotes the rate at which a repres-
Repressor number fluctuations. While the lacI gene is expressed constitutively, fluctuations in the number of lacI-mRNA lead to fluctuations in the number of repressors. Since the transcription rate $c_R$ of the lacI gene is much smaller than the mRNA degradation rate $\phi$ (see SI Table 1), we assume that there is at most one mRNA molecule in the cell. This approximation enables setting up a Fokker–Planck equation for the repressor number using a procedure identical to that used earlier to derive Eq. (3). The resulting drift and diffusion of the repressor numbers $R$ are

\[
\begin{align*}
A_R &= s \frac{c_R l R}{\phi} - R \phi, \\
B_R &= s \frac{(l R)^2}{\phi^2} + R \phi + s^2 \frac{2c_R \phi (l R)^2}{(c_R + \phi) \Gamma},
\end{align*}
\]

where $\phi$ is the total rate of mRNA dilution and degradation, while $c_R$ and $l R$ are the transcription and translation rates for the LacI protein (see SI Table 1). The factor $s = 1/4$ reflects the fact that repressors consist of 4 LacI proteins each. Figure 5 compares the distribution of repressor numbers resulting from (14-15) to the distribution observed in simulations of our detailed mechanistic model and finds good agreement between them.

Setting up the diffusion model. Drift and diffusion of the pump numbers given by equations (4) and (5) depend on the repressor unbinding rate $\eta$ and the binding rate $\beta$. The unbinding rate depends on the pump copy number via equations (11) and (12), and the binding rate fluctuates along with the number of repressors given by equations (14) and (15). Putting these results together gives a bivariate Fokker–Planck equation that describes the joint time evolution of protein numbers $Y$ and repressor numbers $R$,

\[
\partial_t \rho(y, R) = -\partial_y A y \rho(y, R) + \frac{1}{2} \partial_y^2 B y \rho(y, R) - \partial_R A_R \rho(y, R) + \frac{1}{2} \partial_R^2 B_R \rho(y, R).
\]

subject to the boundary conditions

\[
\Gamma(y^{OFF}) = 0, \quad \frac{d T}{d t} \bigg|_{y^{ON}} = 0.
\]

These boundary conditions state that mean first passage time is 0 when the dynamics starts already at the destination $Y^{OFF}$, while near the induced state $Y^{ON}$, the first passage time is insensitive to small perturbations in the starting point. Inducer numbers in the cell at start and end points do not affect the first passage time since they quickly reach the steady state determined by the number of pump proteins given by Eq. (12).

To determine the mean first passage time, we solve the backward Fokker–Planck equation (17) numerically. Fig. 6 shows the results as a function of the external inducer concentration. We compare the first passage times to the induced state obtained from Eq. (17) to simulations of the detailed mechanistic model and find a good match between the diffusion model and the detailed mechanistic model. We find that the mean first passage time increases exponentially with external inducer concentration once the induced state is viable. As a result, for concentrations larger than approximately $10 \mu M$, the induced state is extraordinarily stable, with mean-first passage times exceeding $10^6$ minutes. The induced state can thus persist over many generations, and is actually transmitted more stably to subsequent generations than genetic information: A generation time of 60 min implies a transition
rate to the uninduced state of $O(10^{-7})$ per generation, compared to a point mutation rate of $O(10^{-6})$ per generation. A similar situation has been found in the dormant state of the λ-phage [4].

On the other hand, at external inducer concentrations below 5µM, there is no long-lived induced state, as the lactose that can be imported at such low external concentrations is not sufficient to deactivate all repressors and sustain an induced state. Thus, even if initial pump numbers are large, they quickly decay and the cells collectively transition to the uninduced state. This dynamics has been called a ‘ballistic transition’ [11].

Fluctuations in the number of repressors contribute in different ways to the transition to the uninduced state. Performing simulations of the detailed mechanistic model at constant number of repressors (with repressor number equal to their mean $R = 10$ under the full model), increases the mean first passage time significantly at high inducer concentrations. Higher-than-average repressor numbers lead to long periods where the lac-genes are transcriptionally silenced, making it easier for the pump number to reach lower levels, which can effectively lower the barrier to be crossed by diffusion.

Comparison with experiments. Due to the long mean-first-passage time, the transition to the uninduced state is challenging to observe experimentally. Specifically, the rapid increase of the MFPT with external inducer concentration means that the transition can only be observed in a narrow window where the induced state is stable but the MFPT is shorter than time scale over which the experiment is performed. The experiments were performed as described in [15]. We found the induced state to be unstable at TMG concentrations below 5µM, and observed a ‘ballistic collapse’ of the entire cell population to the uninduced state. At concentrations greater than 10µM, we did not observe any transitions over 8 hours in a population of approximately $10^9$ cells, implying that the mean first passage time is greater than $2 \times 10^3$ minutes (see Fig 6 inset). On the other hand, at an intermediate concentration of 7.5µM, we did observe transitions to the uninduced state occurring at a rate of $1.08 \times 10^{-3}$/min. This behavior qualitatively matches the numerical simulations. For a quantitative comparison far higher population sizes of cells would be needed to observe more transitions to the uninduced state even when the MFPT is large.

**DISCUSSION**

In this paper we have identified the fluctuations that drive the transition to the uninduced state of the lac pathway of *E. coli*. The repressor-operator binding/unbinding, inducer-repressor binding/unbinding, and fluctuations in the total number of repressors all contribute to the stochastic transition between these two states. To make this system tractable, we compute effective rates of repressor binding and dissociation from DNA as functions of pump numbers, and use these in
a bivariate Fokker-Planck equation that captures the stochastic dynamics of pump and repressor copy numbers. From this equation, we compute the mean first passage time to the uninduced state and compare the result to numerical simulations of a detailed mechanistic model.

The transition mechanism is thus the diffusive crossing of a barrier. This is different from the mechanism we previously found for the reverse transition in the same system, the transition from the uninduced to the induced state. There, the key step turned out to be the unbinding of the lac-repressor for a time period exceeding a particular critical duration [15].

These different types of mechanisms have previously been discussed [18] as two distinct possibilities in which gene expression fluctuations can lead to phenotypic switching. It is interesting to see both of them realized in a single, well-studied system.

We find that the barrier-crossing mechanism can give the induced state a remarkable stability: the mean-first-passage-time increases exponentially with the external inducer concentration. As a result, stochastic transitions to the uninduced state can occur at rates as low as $10^{-8}$ per minute, and are thus hard to observe experimentally. Also, the transition to the uninduced state is much slower than the reverse transition to the induced state [15]. A similar asymmetry in the transition rates is found as well in the arabinose uptake network of *E. coli* [35]. However, using the natural inducer lactose in this system would make the induced state less stable (because the inducer is degraded by LacZ), possibly even to the point where the bistability is lost [36].

If the imbalance between the rates of the two transitions persists, it clearly limits the usefulness of stochastic transitions between phenotypic states as a ‘bet hedging’ strategy: It has been proposed that bacteria use stochastic transitions to ensure that at any point in time, part of a population of cells is in one state, part in the other. Depending on external conditions, one state will confer a fitness advantage compared to the other; with stochastic transitions between states, part of the population will always be in the advantageous phenotypic state, no matter how external condition change over time (for a review, see [32]). However, if one rate dominates over the other, nearly all cells will be in only one of the two states. Hence, in the case of the *lac* pathway, stochastic transitions between long-lived states may simply be an unavoidable consequence of implementing bi-stability in a system containing stochastic components, not a feature that confers an evolutionary advantage.

### Competing interests

The authors declare that they have no competing interests.

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