What makes the \textit{lac}-pathway switch: identifying the fluctuations that trigger phenotype switching in gene regulatory systems - Supplementary Information

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1 Experiments

To characterize the switching behavior of the \textit{lac} system, single-cell experiments based on flow cytometry were performed as described in the main text. To determine the switching rate from the uninduced to the induced state, cells were first grown overnight for approximately 12 generations in the absence of the inducer (TMG). Then cells were adapted to a state of steady state exponential growth by re-diluting the growth culture to optical density (OD) 0.04 every hour for four hours, resulting in the pre-culture (Fig. S1). OD values were thus kept in a narrow range
between 0.03 and 0.08. Cells from the pre-culture were transferred to media with different inducer concentrations and cultivated in constant conditions of exponential growth. Cell cultures were diluted by a factor 2 per hour since the strain CH458 doubled in 60 minutes under our experimental conditions (Fig. S2).

We also looked at the switch from the induced state to the uninduced state, by growing cells overnight and pre-culturing them at an inducer concentration of 250 µM as described above for switching to the induced state. However, the induced state is very stable and we only observed switching to the uninduced state in a very narrow range of low inducer concentrations. For this reason, we defer an analysis of the switch to the uninduced state to future work.

Cell populations were grown at various inducer concentrations between 0 µM and 250 µM. Growth cultures were analyzed by flow cytometry on an hourly basis (additional 0.5 hour steps during the first two hours). For a range of inducer concentrations between 10 µM to 200 µM we found two peaks in the fluorescence distribution of the population, corresponding to the uninduced and to the induced expression state respectively (see Fig. 2B of the main text). The fluorescence intensity distribution measured at different intervals yields the fraction of induced cells at various time points, for each value of external TMG concentration we probed. An example of this data evaluation approach is shown in Fig. 2 of the main text. Fitting an exponential curve to the development of the fraction of uninduced cells (using the Gauss-Newton algorithm) results in the switching rates at each inducer concentration as shown in the experimental switching rate curve in Fig. 4 of the main text.

1.1 Growth rates

To probe the possibility that switching rates are influenced by differences in growth rates between the induced and uninduced cells, we monitored the doubling times of cells in our experiments. As shown in Fig. S2, the doubling times are identical at various inducer concentrations. Since cells in high concentrations of inducer are in the induced state while those in low concentrations are in the uninduced state when the growth rates are measured, we conclude that under our experimental conditions the growth rates are identical in the induced and uninduced state. Nevertheless growth rates can affect switching behaviour [1]. Overnight cultivations (leading to a population no longer growing exponentially) in presence of 10 µM TMG for example displayed a fraction of cells in the induced state in contrast to assays at narrow OD windows where the entire population remained in the uninduced state (see Fig. 5).

2 A mechanistic model of the lactose-uptake pathway

The lactose uptake pathway of *E. coli* is a bi-stable regulatory switch that – in the absence of the cell’s primary food source (glucose) and presence of an alternate food source (lactose) – activates mechanisms to import and catabolize lactose. We set up a detailed fully stochastic mechanistic model of the lactose utilization network in *E. coli* in the absence of glucose. This model describes the expression of mRNA and protein (both of the importer LacY and the repressor (LacI tetramer)), the uptake of external inducers into a cell (including passive diffusion), repressor binding to DNA, DNA looping, as well as cell division. These processes will be described in detail below. The Gillespie algorithm [2] was used to simulate its stochastic dynamics at different concentrations of the external inducer. From simulations of a population of such cells, we determined the switching rates between the uninduced and induced states (low/high levels of LacY), by fitting an exponential curve to the fraction of uninduced cells as for the experimental data.

The detailed mechanistic model is specified by a large number of parameters, like transcription and translation rates, binding rates, etc. With a single exception (the rate of inducer import, see below), we took the numerical values of these parameters from the literature in order to avoid overfitting. Unavoidably, those literature sources are uneven in their currentness and accuracy. However, as a result of our analysis, we find that out of the large number of parameters listed in Table SI 1, the switching rate depends only on the 4 parameters entering equation (2) of the main text. These are the parameters linked to the rate limiting fluctuations, namely the operator staying
Figure S1: **Experimental workflow.** Cultures were grown for 16 hours overnight at concentrations of 0 and 250 µM TMG, respectively. As described in the main text, pre-cultures were diluted to the optical density (OD) 0.04 and cultivated for additional four hours with hourly re-dilutions. Finally, the medium was exchanged with an M9 medium containing the destination inducer concentrations in a range from 0 to 250 µM TMG.

free of repressors for a specific time period, see section 4.

### 2.1 Comparison to previous models

Modeling the dynamics of the lac-system, Mettetal et al. [3] formulate a deterministic model of the lac-system which they fit to experiment using several fitting parameters (using the deterministic framework published earlier in [4]) and then experimentally estimate protein number fluctuations and burst sizes (number of proteins produced per mRNA molecule) for LacY, LacI and GFP. Using these parameters, they formulate a stochastic model consisting of bursts of protein production (mRNA production followed by instantaneous protein production and mRNA degradation), protein degradation and extrinsic noise which is modeled using a single noise parameter. Repressor binding to regulatory DNA is not modelled explicitly. However, Choi et al. [5] show that repressor dissociation from its binding sites on DNA plays a crucial role in the switch from the uninduced to the induced state.

Roberts et al. [6] construct a detailed spatially resolved model of the lac-operon. They examine the switching behavior and the effects of such phenomena as spatial crowding. Their results are consistent with the findings of Choi et al. but they further conclude that both mRNA and protein thresholds are crossed during the phenotypic switch.

Stamatakis et al. [7] also construct a detailed, computational, spatially homogeneous stochastic model of the lac-operon, including sources of stochasticity such as cell division, operator fluctuations, mRNA and protein number fluctuations and the effect of dilution. They focus on describing mathematically the effects of this stochasticity at the single cell level on population dynamics and compare it with a deterministic model of the lac-system.

None of the previous models have attempted to identify the rate limiting fluctuation of the switch to the induced
Figure S2: This graph shows the optical density of cells just before re-dilution after 1 hour of cultivation at different concentrations of TMG. Most cells in 250 µM TMG assays have switched to the induced state in the course of the assay while all cells in 0 µM TMG assays remained in the un-induced state. The curves show that there is no significant difference in growth rates in between assays and also over time.

We do not model the spatial heterogeneity of the bacterial cell. Experiments by Kuhlman and Cox [8] show that repressor concentration – and thus the binding probability – depends on the distance between the lacI genes and the lac-operon. As this distance is fixed in our experiments, a constant rate of binding per repressor molecule is appropriate (this rate would change if the distance between genes were changed). A different effect arising from spatial heterogeneity is that a particular repressor molecule that has just been released from the regulatory region might rebind more quickly than one of the other repressor molecules in the system, on account of its spatial proximity to the regulatory region. This is a competition between spatial proximity to the regulatory region (of the originally bound repressor) and the larger number of other repressors (potentially more distant, but more numerous). Roberts et al. [6] estimate the probability that a given repressor molecule binding to the regulatory region is the same molecule that has just unbound (rebinding probability) to be in the range of 0.15 and 0.24, comparable to the result under perfect mixing (0.1 for 10 repressor molecules). This suggests that spatial effects do not make a large difference to the rebinding probability of a repressor that has just dissociated from DNA. In our case, this effect would lead to an increased binding rate that would be absorbed into the one fitting parameter of our model, see section 4.

2.2 Import of sugar into the cell

We refer to the concentration of TMG present outside the cell as eTMG and the number of sugar molecules inside the cell as iTMG. TMG can enter the cell in two ways: (i) active import by importers LacY and (ii) passive
Figure S3: Four different conditions are shown; the assays start with uninduced cells (0 µM TMG). The destination TMG concentration is indicated on top of each graph. Time points displayed are taken at 0, 2, 4, 6 and 8 hours respectively. Since our flow cytometry data of weakly fluorescing cells is unevenly distributed due to machine biases (stretches of consistently empty reads in the first 300 of 1024 channels and culmination in the first channel), events in the low range were fitted to a normal distribution (centered at channel 225 with a standard deviation of 50) for visual clarity.

diffusion. For sugar transport by importers we can write

$$e^{TMG} \xrightarrow{f_1(Y)} i^{TMG},$$

(1)

where \( Y \) denotes the number of LacY molecules in the cell. We base our model of active sugar transport (characterized by the function \( f_1 \)) on [9], where the transport of a lactose-like sugar by importers is found by \textit{in vitro} measurements to follow a hyperbolic function

$$f_1(Y) = \alpha_{tr} \cdot \frac{e^{TMG}}{i_0 + e^{TMG}} \cdot Y.$$  

(2)

By matching the switching rates from our computational model to the switching rates derived from experiment, we estimate the strength of sugar import per pump \( \alpha_{tr} = 9.44/min \). This is the only free parameter of our model. Our fit falls well within the range of values for \( \alpha_{tr} \) reported in the literature (\( \alpha_{tr} = 2.23/min \) [9] to \( \alpha_{tr} < 3000/min \) [10]). In the induced state, the concentrations of inducer inside the cell are about \( 10^4 \) times the concentrations outside [11] which is consistent with our estimate for \( \alpha_{tr} \). We take \( i_0 = 240 \, \mu M \) from [9].
For passive diffusion of sugars in and out of the cell we have

$$eTMG \xrightarrow{\sigma_{in}} iTMG \quad (3)$$

$$iTMG \xrightarrow{\sigma_{out}} eTMG \quad (4)$$

We take $\sigma_{in} = 0.14(\mu M \cdot min)^{-1}$ based on [12]. Using the cellular dimensions of *E. coli* from [13, 14] we estimate the surface area of *E. coli* to be $\approx 4 \times 10^{-12} m^2$ while *E. coli* cell volume is taken to be $6 \times 10^{-19} m^3$[13]. Using these, we can calculate the rate of sugar diffusing out (per sugar molecule in the cell) as $\sigma_{out} = 3.4 \times 10^{-4}/min$.

### 2.3 Repressor production

When transcribed, the *lacI* gene produces the messenger RNA $mRNA_I$ that when translated produces the LacI protein, the monomer of the tetrameric repressor that can bind to the *lac*-regulatory region.

$$\varnothing \xrightarrow{r_{tp}[lacI]} mRNA_I \quad (5)$$

$$\varnothing \xrightarrow{r_{tr}[mRNA_I]} LacI. \quad (6)$$

Measurements reported in [15] indicate that the typical *E. coli* cell produces around 10 complete repressors in one cell cycle. Since the repressor is a tetramer, we have around 40 monomers in the cell. We denote the rate of mRNA degradation by $\alpha_{pm}$. Based on mRNA lifetimes reported in [16], we have $\alpha_{pm} = 0.000666 min$. The burst sizes (the ratio of translation rate to mRNA degradation rate) and burst frequencies (ratio of transcription rate to dilution rate) for the *lac*-operon and other genes in *E. coli* have been extensively studied, for example in [17, 18, 19, 20, 21]. We take a burst size of 5 and burst frequency of 8 (estimated for a low abundance protein) from [18, 21] to obtain the transcription rate ($r_{tp} = 0.1336/min$) and the translation rate ($r_{tr} = 3.33/min$) for *lacI*.

Using the symbol LacI$_2$ for the dimer, and LacI$_4$ for the tetrameric repressor, we represent the tetramerization process through the following reactions

$$LacI + LacI \xrightarrow{r_{di}} LacI_2 \quad (7)$$

$$LacI_2 \xrightarrow{r_{mo}} LacI + LacI \quad (8)$$

$$LacI_2 + LacI_2 \xrightarrow{r_{te}} LacI_4 \quad (9)$$

$$LacI_4 \xrightarrow{r_{td}} LacI_2 + LacI_2. \quad (10)$$

While the rates at which the tetramerization proceeds are not well known, the affinity of the dimer to tetramer reactions is known to be $10^8/M$ [22]. Thus, it is reasonable to expect that most LacI molecules will occur as the tetrameric repressor LacI$_4$ rather than in the monomeric or dimeric states. To reflect this, we take the association rates $r_{di} = r_{te} = 10^3/min$ and the dissociation rates $r_{mo} = r_{td} = 10^{-5}/min$.

### 2.4 Importer production

TMG is imported into the cell by the LacY protein acting as a importer (pump). LacY is produced by a *lac*-gene controlled by the *lac*-regulatory region of the *lac*-genes we refer to as Plac. It is known that the repressor can form a DNA loop by binding to two operator sites, thus effectively hindering transcription of the *lac*-genes [5]. We refer to a *lac*-regulatory region with the DNA-loop formed as Plac2.
Using the measurements from [20], which indicate that the number of LacY proteins in the cell (if there are no active repressors present) is 10000, we take the rates of lacY transcription $y_{tp} = 6/\text{min}$ and translation $y_{tr} = 20/\text{min}$ to obtain a burst size (ratio of translation rate to mRNA degradation rate) of 30 consistent with [17, 23].

However, when the repressor is bound to DNA forming the repressor-DNA loop (the Plac2 state), one end of the repressor can come undone causing the DNA loop to open, leading to leaky production of importers [5], a state we refer to as Plac1. With the lac-genes repressed, the cell is expected to have approximately 10 importer proteins [20], giving a transcription rate of $y_{tp1} = 6 \times 10^{-3}/\text{min}$.

$\emptyset \xrightarrow{y_{tp}[Pla]} mRNAY$ (11)

$\emptyset \xrightarrow{y_{tp}[Pla1]} mRNAY$ (12)

$\emptyset \xrightarrow{y_{tr}[mRNAY]} \text{LacY}$ (13)

Here, the $mRNAY$ is the messenger RNA that when translated produces the importer protein LacY. The degradation rate of mRNA is taken to be $\alpha_{m} = 0.6666/\text{min}$ from [16]

$mRNAY \xrightarrow{\alpha_{m}} \emptyset$ (14)

$mRNAY \xrightarrow{\alpha_{m}} \emptyset$. (15)

### 2.5 Inducer-repressor-DNA interactions

Once the sugar (lactose, here TMG) has been imported into the cell it can bind to the repressor at four sites, one on each monomer. The sugar-bound repressors are less effective at curbing production of importers (see below) and thus the sugar is also referred to as an inducer (of importer production).

We call the complex formed by the repressor with one inducer molecule LacI3s, with two inducer molecules LacI2s, and so on. The complex with four inducer molecules and the repressor we refer to as LacI0s. We take $s_b$ to be the rate at which an inducer binds to a repressor molecule with four free binding sites, while $s_d$ is the dissociation rate of one inducer molecule from its binding site on the repressor.

$LacI4 + \text{iTMG} \xrightarrow{s_b} LacI3s$ (16)

$LacI3s + \text{iTMG} \xrightarrow{s_b} LacI2s$ (17)

$LacI2s + \text{iTMG} \xrightarrow{s_b} LacI1s$ (18)

$LacI1s + \text{iTMG} \xrightarrow{s_b} LacI0s$ (19)

$LacI0s \xrightarrow{s_d} LacI1s + \text{iTMG}$ (20)

$LacI1s \xrightarrow{s_d} LacI2s + \text{iTMG}$ (21)

$LacI2s \xrightarrow{s_d} LacI3s + \text{iTMG}$ (22)

$LacI3s \xrightarrow{s_d} LacI4 + \text{iTMG}$. (23)

The rates of inducer-repressor binding and unbinding were investigated in vitro by Dunaway et al. [24]. They give the binding rate as $s_b = 2.29 \times 10^{-3}/\text{min}$ and the dissociation rate as $s_d = 12/\text{min}$.

Dunaway et al. also find that interactions between inducers and DNA-bound repressors are weaker than the interactions between inducers and free repressors. In particular, DNA-bound repressors bind to inducers 4.6 times more slowly, and dissociate 4 times faster. We represent the DNA-repressor-inducer complex by $Pla_{1,\text{rep}}$ if the
repressor is bound to one operator site and $Plac_{2, rep}$ if the repressor is bound to two operator sites forming a DNA loop. The # stands for the number of unoccupied inducer binding sites on the repressor. We take $b_i$ to be the rate at which an inducer binds to an inducer free repressor bound to one binding site on the DNA, while $d_i$ is the dissociation rate of an inducer molecule from its binding site on a DNA bound repressor. $d_i$ follows from the detailed balance condition that the inducer binds to a repressor which is part of a repressor-DNA loop at a rate $b_{i2}$ different from $b_i$ (see Section 2.5.1 below).

$$Plac_{1,rep} + iTMG \xrightarrow{b_i} Plac_{1,rep3}$$  \hspace{1cm} (24)
$$Plac_{1,rep3} + iTMG \xrightarrow{3b_i} Plac_{1,rep2}$$  \hspace{1cm} (25)
$$Plac_{1,rep2} + iTMG \xrightarrow{b_f} Plac_{1,rep1}$$  \hspace{1cm} (26)
$$Plac_{1,rep1} + iTMG \xrightarrow{b_i} Plac_{1,rep0}$$  \hspace{1cm} (27)
$$Plac_{2,rep4} + iTMG \xrightarrow{b_{i2}} Plac_{2,rep3}$$  \hspace{1cm} (28)
$$Plac_{2,rep3} + iTMG \xrightarrow{3b_2} Plac_{2,rep2}$$  \hspace{1cm} (29)
$$Plac_{2,rep2} + iTMG \xrightarrow{b_2} Plac_{2,rep1}$$  \hspace{1cm} (30)
$$Plac_{2,rep1} + iTMG \xrightarrow{b_{i2}} Plac_{2,rep0}$$  \hspace{1cm} (31)
$$Plac_{1,rep0} \xrightarrow{4d_f} Plac_{1,rep1} + iTMG$$  \hspace{1cm} (32)
$$Plac_{1,rep1} \xrightarrow{3d_i} Plac_{1,rep2} + iTMG$$  \hspace{1cm} (33)
$$Plac_{1,rep2} \xrightarrow{2d_i} Plac_{1,rep3} + iTMG$$  \hspace{1cm} (34)
$$Plac_{1,rep3} \xrightarrow{d_1} Plac_{1,rep4} + iTMG$$  \hspace{1cm} (35)
$$Plac_{2,rep0} \xrightarrow{4d_f} Plac_{2,rep1} + iTMG$$  \hspace{1cm} (36)
$$Plac_{2,rep1} \xrightarrow{3d_i} Plac_{2,rep2} + iTMG$$  \hspace{1cm} (37)
$$Plac_{2,rep2} \xrightarrow{2d_i} Plac_{2,rep3} + iTMG$$  \hspace{1cm} (38)
$$Plac_{2,rep3} \xrightarrow{d_1} Plac_{2,rep4} + iTMG.$$  \hspace{1cm} (39)

Dunaway et al. measure $s_d$, $d_i$, $b_1$, $s_b$, while the rate $b_{i2}$ is set by detailed balance (see Section 2.5.1).

$$s_d = 12/\text{min}$$
$$d_i = 48/\text{min}$$
$$b_1 = 4.98 \times 10^{-4}/\text{min}$$
$$s_b = 2.29 \times 10^{-3}/\text{min}.$$  

The interactions between the lac-regulatory region and the repressor depend on the number of inducers bound to the repressor. In general, repressors with different number of inducers bound to them might bind and unbind from the lac-regulatory region at different rates. However Winter et al. [25] find that inducer binding states do not significantly change the rate of repressor-lac-regulatory region binding. This is consistent with the idea that the binding rate of the repressor to the operator ($g_r$) is limited by the 3D and 1D random searches that the repressor
conducted to find its binding site [26, 27]. Thus, we have
\[
\begin{align*}
\text{LacI}_4 + \text{Plac} &\rightarrow \text{Plac}_{\text{rep}4} \\
\text{LacI}_3s + \text{Plac} &\rightarrow \text{Plac}_{\text{rep}3} \\
\text{LacI}_2s + \text{Plac} &\rightarrow \text{Plac}_{\text{rep}2} \\
\text{LacI}_1s + \text{Plac} &\rightarrow \text{Plac}_{\text{rep}1} \\
\text{LacI}_0s + \text{Plac} &\rightarrow \text{Plac}_{\text{rep}0}.
\end{align*}
\]

The binding rate of a single repressor to its binding sites has been measured in [28, 20] and they put an upper bound of 6 minutes for a single repressor to find one of its binding sites. This corresponds to \( g_r = 0.166/\text{min} \).

We represent the dissociation rate of a repressor with \( m \) free binding sites (\( 4 - m \) inducers bound) from one of its binding sites on the DNA by \( d_m \).

\[
\begin{align*}
\text{Plac}_{\text{rep}0} d_0 \rightarrow \text{Plac} + \text{LacI}_0s \\
\text{Plac}_{\text{rep}1} d_1 \rightarrow \text{Plac} + \text{LacI}_1s \\
\text{Plac}_{\text{rep}2} d_2 \rightarrow \text{Plac} + \text{LacI}_2s \\
\text{Plac}_{\text{rep}3} d_3 \rightarrow \text{Plac} + \text{LacI}_3s \\
\text{Plac}_{\text{rep}4} d_4 \rightarrow \text{Plac} + \text{LacI}_4.
\end{align*}
\]

When one end of the repressor is bound, the rate at which the other end binds to a second site to form the repressor-DNA loop is represented by \( g_l \). Either of two ends of the repressor can unbind from the DNA to open the loop. DNA loop formation is then described by

\[
\begin{align*}
\text{Plac}_{\text{rep}4} g_l \rightarrow \text{Plac}_{\text{rep}0} \\
\text{Plac}_{\text{rep}3} g_l \rightarrow \text{Plac}_{\text{rep}2} \\
\text{Plac}_{\text{rep}2} g_l \rightarrow \text{Plac}_{\text{rep}2} \\
\text{Plac}_{\text{rep}1} g_l \rightarrow \text{Plac}_{\text{rep}1} \\
\text{Plac}_{\text{rep}0} g_l \rightarrow \text{Plac}_{\text{rep}0} \\
\text{Plac}_{\text{rep}2} 2d_0 \rightarrow \text{Plac}_{\text{rep}0} \\
\text{Plac}_{\text{rep}1} 2d_1 \rightarrow \text{Plac}_{\text{rep}1} \\
\text{Plac}_{\text{rep}2} 2d_2 \rightarrow \text{Plac}_{\text{rep}2} \\
\text{Plac}_{\text{rep}3} 2d_3 \rightarrow \text{Plac}_{\text{rep}3} \\
\text{Plac}_{\text{rep}4} 2d_4 \rightarrow \text{Plac}_{\text{rep}4}.
\end{align*}
\]

The rate \( d_4 = 2.4/\text{min} \) (the dissociation rate of repressor molecule from its binding site) has been measured by Dunaway et al. in [24]. The rates \( d_3, d_2, d_1, d_0 \) are determined by the condition of detailed balance as described in Section 2.5.1.

The rate at which the repressor unbinds from both its binding sites and dissociates completely from the lac-regulatory region is estimated in [20] to be \( d_t \approx 0.025/\text{min} \). Following the reasoning of Choi et al., total unbinding of the repressor from the DNA occurs when there are two consecutive unbinding events. The repressor-DNA loop is broken by the first unbinding event and if the second unbinding event occurs before the loop is re-formed, the
repressor completely unbinds from the lac-regulatory region. The rate of complete dissociation of the repressor \((d_t)\) from the DNA depends on the rate at which the repressor unbinds from one of its binding sites \((d_4)\) and the rate at which the loop reforms if the repressor is bound to one operator site represented by \(g_l\). As discussed earlier in this section, \(d_4\) and \(d_t\) have been measured in [24, 20]. To compute \(g_l\), we must determine the relationship between \(d_t, g_l\) and \(d_4\).

We assume that the two ends of the repressor bound to DNA unbind independently of each other at rate \(d_4\) and that successive unbinding events are also independent of earlier binding, unbinding events. Thus, we can treat the unbinding of the repressor from each of its binding sites as independent Poisson processes both characterized by the rate \(d_4\). Similarly, we can treat the re-formation of the loop when one end of the repressor is bound to DNA as a Poisson process characterized by the rate \(g_l\).

The time intervals \(t\) between successive events in a Poisson process with a rate parameter \(\gamma\) are distributed exponentially as \(p(t) = \gamma e^{-\gamma t}\). If the repressor is bound to one operator site, two processes are possible:

1. Repressor unbinds from its binding site and leaves the DNA at rate \(d_4\),

2. Repressor binds to a second operator site resulting in the formation of a DNA loop at rate \(g_l\).

In this situation, let \(t_{b2}\) be the time interval between a single unbinding event opening the DNA loop (resulting in the repressor being bound to DNA only at one end) and a successive DNA loop re-formation event. Similarly, let \(t_u\) be the time interval between opening of the DNA loop (as before) and a successive unbinding event that results in complete dissociation of the repressor from the lac-regulatory region. Let \(p_{b2}(t_{b2})\) be the probability distribution of time to DNA loop formation and \(p_u(t_u)\) be the probability distribution of time to repressor unbinding. Then we have

\[
p_{b2}(t_{b2}) = g_l e^{-g_l t_{b2}} \tag{60}
\]
\[
p_u(t_u) = d_4 e^{-d_4 t_u}. \tag{61}
\]

For a well behaved function \(f(t)\) the Laplace transform \(F(s)\) is defined as

\[
F(s) = \int_0^\infty f(t)e^{-st}dt. \tag{62}
\]

In the Laplace domain we have

\[
P_{b2}(s) = \frac{g_l}{g_l + s} \tag{63}
\]
\[
P_u(s) = \frac{d_4}{d_4 + s}. \tag{64}
\]

The DNA loop forms when the repressor is bound to two sites on the DNA. Unbinding from either of these sites (each at rate \(d_4\)) results in the opening of the DNA loop. If \(t_{2u}\) is the time interval between DNA loop formation and the first unbinding event that opens the DNA loop, the probability distribution of \(t_{2u}\) is given by

\[
p_{2u}(t_{2u}) = 2d_4 e^{-2d_4 t_{2u}} \tag{65}
\]
\[
P_{2u}(s) = \frac{2d_4}{2d_4 + s}. \tag{66}
\]

After one unbinding event has taken place on a DNA-repressor loop, the repressor will stay bound to DNA if time to the second binding event is shorter than the time to the second unbinding event. The probability of this can be written as
\[
P(t_{b2} < t_u) = \int_0^\infty P_{b2}(t < t_u)P_u(t_u)dt_u \quad (67)
\]

\[
= \int_0^\infty dt_u \left[ \int_0^{t_u} p_{b2}(t)dt \right] P_u(t_u) \quad (68)
\]

\[
= \int_0^\infty dt_u \left[ \int_0^{t_u} gte^{-gt}dt \right] P_u(t_u) \quad (69)
\]

\[
= \int_0^\infty dt_u (1 - e^{-gt_u}) d_4 e^{-d_4 t_u}. \quad (70)
\]

This yields

\[
P(t_{b2} < t_u) = \frac{g_t}{g_t + d_4}. \quad (71)
\]

If \( p_{cy}(n) \) is the probability that \( n \) such cycles (of the repressor unbinding from one end and then rebinding to the DNA) happen before total unbinding, we have

\[
p_{cy}(n) = \left[ \frac{g_t}{g_t + d_4} \right]^n \frac{d_4}{g_t + d_4}. \quad (72)
\]

Since the time taken by each unbinding-rebinding cycle \((t_{cy})\) is given by the addition of two independent random variables \((t_{2u} \text{ and } t_{b2})\) with known distributions \(p_{2u}(t_{2u})\) and \(p_{b2}(t_{b2})\), the distribution of \(t_{cy}\) can be written (in the Laplace domain) as

\[
P_{cy}(s) = P_{2u}(s)P_{b2}(s) \quad (73)
\]

\[
= \frac{2d_4}{2d_4 + s} \cdot \frac{g_t}{g_t + s}. \quad (74)
\]

The probability generating function (PGF) \(G(s)\) for a non-negative discrete random variable \(n\) with a probability distribution \(p(n)\) is defined as

\[
G(s) = \sum_{n=0}^{\infty} p(n)s^n. \quad (75)
\]

Thus for the PGF of \(p_{cy}(n)\) we have

\[
G_{cy}(s) = \left[ \frac{d_4}{g_t + d_4} \right] \sum_{n=0}^{\infty} \left[ \frac{g_t}{g_t + d_4} \right]^n s^n \quad (76)
\]

\[
= \frac{d_4}{g_t + d_4} \cdot \frac{1}{1 - s \cdot \frac{g_t}{g_t + d_4}}. \quad (77)
\]

We can compute the average number of times \(E(n)\) such a cycle (i.e repressor unbinding from one end and then rebinding to the DNA) takes place before total unbinding

\[
E(n) = \left[ \frac{d}{ds} G_{cy}(s) \right]_{s \rightarrow 1} = \frac{g_t}{d_4}. \quad (78)
\]

We can also compute the expectation value of the time taken by each cycle \(E(t_{cy})\)

\[
E(t_{cy}) = \left[ \frac{d}{ds} P_{cy}(s) \right]_{s \rightarrow 0} \quad (79)
\]

\[
= \frac{1}{2d_4} + \frac{1}{g_t}. \quad (80)
\]
Starting from the DNA-repressor loop, the expectation value of the complete unbinding time $E(t_{dub})$ is

$$E(t_{dub}) = E(t_{cy}) \cdot E(n) + E(t_{2u})$$

$$= \frac{g_t}{d_4} \left[ \frac{1}{2d_4} + \frac{1}{g_t} \right] + \frac{1}{2d_4}. \quad (81)$$

This gives the rate of total unbinding $d_t$

$$\frac{1}{d_t} = \frac{g_t}{d_4} \left[ \frac{1}{2d_4} + \frac{1}{g_t} \right] + \frac{1}{2d_4}. \quad (82)$$

Writing $g_t$ in terms of $d_t$ and $d_4$ we obtain

$$g_t = 2d_4 \left[ \frac{d_4}{d_t} - \frac{3}{2} \right]. \quad (83)$$

Substituting values of $d_t$ and $d_4$ we calculate $g_t = 453.6$.

### 2.5.1 Detailed balance

In a system composed of many elementary processes (such as chemical reactions), the principle of detailed balance applies when every elementary process is time reversible. At equilibrium, this microscopic reversibility implies that every elementary process must be balanced by its reverse process. While detailed balance does not apply to irreversible processes such as transcription and translation, we can use it to constrain rates in reaction networks involving binding and unbinding of ligands and their substrates. Tree-like reaction networks (which do not have any cycles) in equilibrium always obey detailed balance. For reaction networks which do include cycles, detailed balance provides a relationship between the rates of the reactions involved in each cycle [29]. As an illustration, consider the following cycle:

$$A_1 \xrightleftharpoons[k_{12}]{k_{21}} A_2 \xrightleftharpoons[k_{23}]{k_{32}} A_3 \ldots A_p \xrightleftharpoons[k_{pq}]{k_{qp}} A_q \ldots A_n \xrightleftharpoons[k_{1p}]{k_{p1}} A_1. \quad (85)$$

Then, the relationship between the rates is given by

$$k_{12}k_{23}\ldots k_{pq}\ldots k_{n1} = k_{21}k_{32}\ldots k_{qp}\ldots k_{1n}. \quad (86)$$

Thus, the principle of detailed balance can be used to determine one rate in each reaction cycle in terms of the others. We consider the reactions involving inducer (TMG) binding to the repressor and the repressor binding to the operator (Eq. (17) to Eq. (60)). The rates of total repressor dissociation $d_t$, repressor dissociation from one binding site $d_4$, inducer binding to repressor bound and unbound from DNA ($b_i, s_b$) and inducer unbinding from repressor bound and unbound from DNA ($d_i, s_d$) have been measured in experiments (see Table S1). Dunaway et al. [24] report that rates at which repressors with one or more inducers bound to them dissociate from DNA ($d_3, d_2, d_1, d_0$), are too fast to measure. We use the detailed balance condition to deduce these rates along with the rate at which inducers dissociate from a repressor bound to the DNA at both ends ($b_{i2}$).

We analyze each cycle in the reaction network defined by Eqns. (17-60) and use Eq. (86) to calculate the rates
\( (d_3, d_2, d_1, d_0, b_{i2}) \) in terms of experimentally measured rates to obtain

\[
d_3 = \frac{s_b d_4}{s_d b_i} = 44.144/\text{min} \quad (87)
\]

\[
b_{i2} = \frac{b_i d_4}{d_3} = 2.707 \times 10^{-5}/\text{min} \quad (88)
\]

\[
d_2 = \frac{s_b d_3}{s_d b_i} = 811.97/\text{min} \quad (89)
\]

\[
d_1 = \frac{s_b d_2}{s_d b_i} = 14935.15/\text{min} \quad (90)
\]

\[
d_0 = \frac{s_b d_1}{s_d b_i} = 274710.82/\text{min}. \quad (91)
\]

### 2.6 Dilution rate

All molecular species inside the cell are subject to dilution via cell growth,

\[
\text{LacY} \xrightarrow{\alpha_b} \emptyset \quad (92)
\]

\[
\text{LacI} \xrightarrow{\alpha_b} \emptyset \quad (93)
\]

\[
\text{LacI2} \xrightarrow{\alpha_b} \emptyset \quad (94)
\]

\[
\text{LacI4} \xrightarrow{\alpha_b} \emptyset \quad (95)
\]

\[
\text{LacI3s} \xrightarrow{\alpha_b} \emptyset \quad (96)
\]

\[
\text{LacI2s} \xrightarrow{\alpha_b} \emptyset \quad (97)
\]

\[
\text{LacI1s} \xrightarrow{\alpha_b} \emptyset \quad (98)
\]

\[
\text{LacI0s} \xrightarrow{\alpha_b} \emptyset \quad (99)
\]

\[
\text{iTMG} \xrightarrow{\alpha_b} \emptyset. \quad (100)
\]

In our experiments the dilution rate is \( \alpha_b = 0.0167/\text{min} \), see Figure S2.

### 2.7 Cell Division

Cell growth and division involve random partitioning of molecules between the daughter cells which is a source of mRNA and protein number fluctuations. If there are \( n \) molecules of a particular type inside the cell just before division and each molecule is equally likely to go to either of the two daughter cells, the probability that \( m \) molecules go to one daughter cell while \( (n - m) \) go to the other is distributed binomially \[30\] as

\[
P_n(m) = \binom{n}{m} 2^{-n}. \quad (101)
\]

Every doubling period (60 minutes, in our case) and for each type of molecule \( (j) \) in our system (sugars, mRNA, proteins) with abundances \( n_j \), we use the distribution given by Eq. (101) to draw a number \( m_j \). In Eq. (101) cell division is assumed to be symmetrical and thus the mean \( \langle m_j \rangle \) is given by \( \langle m_j \rangle = n_j/2 \). To normalize the number of molecules per unit volume (the daughter cell has half the volume of the mother cell) we take the abundance of molecule \( (j) \) in the daughter cell to be \( 2m_j \). The variance in \( m_j \) implied by Eq. (101) is \( n_j 2^{-2} \).
Since the variance is proportional to abundance \( n_j \), the coefficient of variance (the ratio of the standard deviation to the mean) is proportional to \( 1/\sqrt{n_j} \) showing that partitioning noise is more significant for molecules with low abundances.

2.8 Delays

Delays between different events in a gene regulatory model can be used to effectively describe mechanisms that are not modeled explicitly. For instance, in a model describing gene expression by a single step (omitting RNA production) one can use a delay between transcription factor binding and protein production to account for the intermediate steps of transcription and translation. Such delays, whose duration is a random variable drawn from some distribution, can affect the switching behavior between phenotypic states [31]. In our approach we aim at first for a highly detailed model, which explicitly includes intermediate steps such as transcription and translation. Each of these steps happens at a finite rate, hence delays e.g. between transcription factor binding and protein production emerge naturally from the model, rather than being put in by hand via an \textit{ad-hoc} delay distribution. It might be that our detailed model is still not sufficiently detailed, requiring the inclusion of either further explicit reactions, or alternatively delays. An example for such a step is the folding of proteins. However, the good match between experimentally determined switching rates and the switching rates observed in the model suggest that we have already captured the relevant processes.

3 The smoothing procedure

As an example, we consider an mRNA molecule produced and degraded in steps of 1 molecule at some rate. The smoothing procedure consists in changing the step size of copy number changes by a factor \( s < 1 \), and simultaneously dividing the transcription and degradation rates by the same factor. For a smoothing factor \( s = 0.1 \), the mRNA molecules are produced in units of 0.1 but at 10 times the original rate.

The effect of this procedure is illustrated in Figure S4. The mean number of molecules is preserved, but the fluctuations about this mean are reduced by a factor of \( s \). Deterministic dynamics corresponds to \( s = 0 \), while finite values of \( s \) result in some degree of stochasticity. If smoothing fluctuations in a particular component affects the switching rate between stable states, we conclude that these fluctuations are rate limiting to the particular switching process.

Such smoothing impossible to do experimentally but feasible \textit{in silico}; the rate of protein production will now simply be proportional to a non-integer number of mRNA molecules, and analogously for other molecules and binding states. In general this leads to non-integer numbers of molecules in the system. Nevertheless, a well-defined stochastic system results from this procedure: for instance, the Gillespie algorithm simulates transitions between states \( a, b, c, \ldots \). For \( s = 1 \) these states correspond to \((0, 1, 2, 3, \ldots)\) molecules in the system, while at \( s = 0.1 \) they are \((0, 0.1, 0.2, 0.3, \ldots)\). The application of this procedure to molecular species like importers and repressors is straightforward, but smoothing the \textit{lac}-regulatory region state requires separate consideration (see Section 3.1).

We also found that the noise introduced by random partitioning at cell division does not play a role in the switch to the induced state. We eliminated the noise from cell division by skipping the random partitioning step (see Section 2.7) and found no effect on the switching rate curve. As has been pointed out earlier (in Section 2.7), fluctuations due to random partitioning are more significant for molecules with low abundances. While there are only about 10 repressors in the \textit{E. coli} cell, the \textit{lac} system is unaffected by changes in repressor numbers as long as a repressor is bound to the \textit{lac}-regulatory region. Since the lifetime of the repressor-DNA loop is large (approximately 40\text{min}) as compared to the time scale of repressor mRNA transcription (approximately 7\text{min}), the switching rate is insensitive to smoothing of repressor number fluctuations including those caused by cell division.
The table below provides the parameters of the model and their literature sources:

| Symbol | Value | Description | Literature Sources |
|--------|-------|-------------|-------------------|
| \( \alpha_{tr} \) | 9.44/\text{min} | max. rate of sugar transport per pump | chosen to obtain best fit to our experiment |
| \( i_0 \) | 240\( \mu \text{M} \) | half max of sugar transport | from [9] |
| \( \sigma_{in} \) | 0.14/\text{min} | diffusion of sugars in | based on [5, 32, 13, 14, 12] |
| \( \sigma_{out} \) | \( 3.4 \cdot 10^{-4}/\text{min} \) | sugar diffusion out | based on [12] |
| \( y_{tp} \) | 6/\text{min} | \( mRNA_{AY} \) prod. gene on | based on [20] |
| \( y_{tp1} \) | \( 6 \cdot 10^{-9}/\text{min} \) | \( mRNA_{AY} \) prod. leak | based on [20, 5] |
| \( y_{tr} \) | 20/\text{min} | translation rate for LacY | based on [20] and burst parameter 30 |
| \( r_{tp} \) | 0.1336/\text{min} | \( mRNA_{Y} \) prod. | based on [15] |
| \( r_{tr} \) | 3.33/\text{min} | Lacl translation | est. by setting burst parameter to 5 |
| \( r_{di} \) | \( 10^{3}/\text{min} \) | Lacl dimerization | est. [22] for fast dimerization |
| \( r_{me} \) | \( 10^{-3}/\text{min} \) | Lacl dimer dissociation | based on [22] |
| \( r_{te} \) | \( 10^{3}/\text{min} \) | Lacl tetramerization | based on [22] |
| \( r_{td} \) | \( 10^{-9}/\text{min} \) | Lacl tetramer dissociation | based on [22] |
| \( s_b \) | \( 2.29 \cdot 10^{-3}/\text{min} \) | TMG-Lacl bind, no DNA | from [24] |
| \( s_d \) | 12/\text{min} | TMG-Lacl diss, no DNA | from [24] |
| \( g_r \) | 0.166/\text{min} | repressor-operator binding | measured in [28] |
| \( g_l \) | 453.6/\text{min} | 2nd repressor-Op binding | set by detailed balance |
| \( d_4 \) | 2.4/\text{min} | repressor-DNA diss, no TMG | measured in [24] |
| \( d_3 \) | 44.14/\text{min} | repressor-DNA diss, 1 TMG | set by detailed balance |
| \( d_2 \) | 811.97/\text{min} | repressor-DNA diss, 2 TMG | set by detailed balance |
| \( d_1 \) | 14935.15/\text{min} | repressor-DNA diss, 3 TMG | set by detailed balance |
| \( d_0 \) | 274710.82/\text{min} | repressor-DNA diss, 4 TMG | set by detailed balance |
| \( b_1 \) | \( 4.98 \cdot 10^{-4}/\text{min} \) | TMG-Lacl bind, DNA | from [24] |
| \( b_{12} \) | \( 2.707 \cdot 10^{-9}/\text{min} \) | TMG-Lacl bind, DNA loop | set by detailed balance |
| \( d_i \) | 48/\text{min} | TMG-Lacl unbind, DNA | from [24] |
| \( \alpha_{\varphi} \) | 0.0167/\text{min} | dilution rate | experiments described in Section. (1) |
| \( \alpha_{\varphi m} \) | 0.0666/\text{min} | Lacl mRNA lifetime | from [20] |
| \( V_{E_{coli}} \) | \( 6 \cdot 10^{-19} \text{m}^3 \) | volume of the \( E. \ coli \) cell | from [13, 14] |
| \( p_{mTMG} \) | \( 2 \cdot 10^{-10} \text{m/min} \) | membrane permeability for TMG | based on [33] |

Table S1: Parameters of the model and their literature sources
Figure S4: The smoothing procedure is illustrated with a birth-death process where some molecule is created and degraded at constant rates. Plot (A) shows the time series of molecular copy numbers with a mean of 10 molecules (red line). For the smoothed dynamics (green line), step sizes are multiplied by the smoothing factor $s = 0.01$ and rates are divided by $s$, leaving the mean unchanged. Plot (B) shows the standard deviation of molecular copy numbers against the smoothing factor $s$. The amplitude of fluctuations around the mean increases with $s$. 
3.1 Smoothing operator state fluctuations

The regulatory region of a gene has only two states with respect to its repressors, occupied and unoccupied. We aim to smooth the fluctuations in the operator state without changing the mean production rate of gene product. For this discussion, we assume that the regulatory region undergoes the transition occupied \( \rightarrow \) unoccupied at a rate \( u \) and the reverse transition at a rate \( b \). If the rate of production of gene product when the operator is unoccupied is \( r \) while the rate of production when operator is occupied by the repressor is approximately zero, the mean rate of production of gene product is \( ru \frac{1}{s} + b \).

To smooth the fluctuations in \( \text{lac} \)-regulatory region state by a factor \( s \), we picture a population of pseudo-promoters which can reach a maximum number of \( \frac{1}{s} \) pseudo-promoters, each producing gene product at a rate \( rs \). Now, we define the birth rate of pseudo-promoters

\[
    u(n) = u \left( \frac{1}{s} - n \right),
\]

where \( n \) is the current population of pseudo-promoters. Note that the birth rate is dependent on \( n \) and goes to 0 as \( n \) approaches its maximum value of \( 1/s \). The death rate is the usual population dependent death rate

\[
    b(n) = bn.
\]

Thus, the mean population of pseudo-promoters is

\[
    n_{\text{mean}} = \frac{u}{u + b}
\]

and the mean rate of production of gene product

\[
    rs \frac{u}{u + b} = r \frac{u}{u + b}
\]

as expected. For \( s \rightarrow 0 \) the fluctuations around the mean tend to zero.

For this binary system (occupied and unoccupied state), a simpler smoothing procedure is also feasible [34], namely to increase of both binding and unbinding rates keeping their ratio constant. However, the limit of this procedure is not a deterministic process, whereas the method based on a population of pseudo-operators generalizes the smoothing described above to binary systems. Nevertheless we expect the two methods to yield identical results in practice.

As noted in the main text, we find that for our system the only relevant fluctuations for the uninduced \( \rightarrow \) induced switch are fluctuations in the operator state. Smoothing the fluctuations in other species does not affect the rate of switching, while smoothing fluctuations in the operator state slows down the rate of phenotypic switching to the point of being unobservable over the timescale of our simulations.

4 Theoretical model of the switch to the induced state

In numerical simulations of the mechanistic model we observe that the \( \text{lac} \)-system switches from the uninduced to the induced state if and only if the \( \text{lac} \)-regulatory region stays free of repressors for a certain threshold period \( \tau \). The time period for which the operator is free of repressors depends on the binding and unbinding rates of repressor to the operator. The rate \( d_t \) at which the repressor completely unbinds from the operator is given by Eqn. (83), while \( g_r \) is the rate at which a single repressor will bind to the operator (SI Section 2). If \( n_r \) is the number of repressors in the cell, then the rate at which the \( \text{lac} \)-regulatory region will be found by some repressor is \( k_b = n_r g_r \). In the \( \text{lac} \)-system there are approximately 10 repressors in the cell [15], giving \( k_b = 10 \times g_r = 1.66 \).

Based on these rates we now calculate the expected time until the \( \text{lac} \)-regulatory region stays free of repressors for some period \( \tau \). In a subsequent step, the threshold period \( \tau \) will be calculated.
4.1 The switching rate

Following the argument made in Section 2.5, we treat binding and unbinding of the repressor from the lac-regulatory region as independent Poisson processes characterized by the rates $d_t$ and $d_t$ respectively. We note again that the time intervals $t$ between successive events in a Poisson process with a rate parameter $\gamma$ are independently distributed according to an exponential distribution $p(t) = e^{-\gamma t}$.

Let $p_b(t_b)$ denote the probability distribution of time periods $t_b$ over which the lac-regulatory region remains occupied by a repressor, i.e. $t_b$ is the time interval between a binding and a successive complete unbinding event. Similarly, we denote by $t_u$ the time intervals for which the lac-regulatory region remains free of repressors, i.e. $t_u$ is the time period between a complete unbinding event and a binding event that follows it. Let the probability distribution of times $t_u < \tau$ for which the lac-regulatory region remains free of repressors for a period less than the threshold period $\tau$ be $p_{t_u < \tau}(t_u)$. We denote the Laplace transform of $p_b(t_b)$ by $P_b(s)$ and the Laplace transform of $p_{t_u < \tau}(t_u)$ by $P_u(s)$. Then we have

$$p_b(t_b) = d_t \cdot e^{-d_t t_b}$$  \hspace{1cm} (106)

$$P_b(s) = \frac{d_t}{s + d_t}$$  \hspace{1cm} (107)

$$p_{t_u < \tau}(t_u) = \begin{cases} 
\frac{k_b \cdot e^{-k_b t_u}}{1 - e^{-k_b}} & \text{if } t_u < \tau, \\
0 & \text{if } t_u > \tau.
\end{cases}$$  \hspace{1cm} (108)

$$P_u(s) = \frac{k_b}{e^{k_b \tau} - 1} \cdot \frac{e^{k_b \tau} - e^{-s \tau}}{k_b + s}.$$  \hspace{1cm} (109)

We now consider a cycle of binding, complete unbinding and rebinding before the threshold period $\tau$ has elapsed, taking the time $t_b$ plus $t_u < \tau$. We denote the Laplace transform of the distribution of the time taken by each cycle ($t_u + t_b$) by $P_{cy}(s)$. Since the time taken by each unbinding-binding cycle is just the addition of two random variables (time intervals over which the operator region is bound $t_b$ and unbound $t_u$) with known distributions $p_b(t_b)$ and $p_{t_u < \tau}(t_u)$, in the Laplace domain we have

$$P_{cy}(s) = P_b(s) \cdot P_u(s).$$  \hspace{1cm} (110)

Using $P_{cy}(s)$ we can calculate the average time taken for one unbinding-rebinding cycle $E(t_{cy})$ (as in Section 2.5)

$$E(t_{cy}) = \left[ \frac{d}{ds} P_{cy}(s) \right]_{s \to 0}$$  \hspace{1cm} (111)

$$= \left[ \frac{1}{k_b} + \frac{1}{d_t} - \frac{\tau}{e^{k_b \tau} - 1} \right]_{s \to 0}.$$  \hspace{1cm} (112)

The number of unbinding-rebinding cycles before the lac-regulatory region finally remains unoccupied for a time greater than $\tau$ is a random number. Let $p_{cy}(n)$ be the probability that $n$ such unbinding-binding cycles occur before the lac-regulatory region finally remains unoccupied for time greater than $\tau$ and let $G_{cy}(s)$ be the corresponding probability generating function (as defined in Section 2.5)

$$p_{cy}(n) = (1 - e^{-k_b \tau})^n \cdot e^{-k_b \tau}$$  \hspace{1cm} (113)

$$G_{cy}(s) = \frac{1}{(1 - e^{-k_b \tau})} \sum_{n=0}^{\infty} (1 - e^{-k_b \tau})^n \cdot e^{-k_b \tau} \cdot s^n$$  \hspace{1cm} (114)

$$= \frac{e^{-k_b \tau}}{(1 - e^{-k_b \tau})} \frac{1}{1 - s \cdot (1 - e^{-k_b \tau})}.$$  \hspace{1cm} (115)
If $E(n)$ is the average number of unbinding-rebinding cycles before the operator region remains unoccupied for a time $\tau$, we can calculate $E(n)$

$$E(n) = \left[ \frac{d}{ds} G_{cy}(s) \right]_{s \to 1}$$

$$= \left[ \frac{e^{-k_b \cdot \tau}}{(1 - e^{-k_b \cdot \tau})} \frac{d}{ds} \left( \frac{1}{1 - s \cdot (1 - e^{-k_b \cdot \tau})} \right) \right]_{s \to 1}$$

$$= \left[ \frac{e^{-k_b \cdot \tau}}{(1 - e^{-k_b \cdot \tau})} \left( \frac{1}{1 - s \cdot (1 - e^{-k_b \cdot \tau})} \right)^2 \right]_{s \to 1}$$

$$= \frac{e^{-k_b \cdot \tau}}{e^{-2k_b \cdot \tau}} = e^{k_b \cdot \tau}. \quad (119)$$

Given that successive binding and unbinding events are statistically independent, the expected time of switching $E(t_{sw})$ is the average time taken by every binding-unbinding-rebinding cycle $E(t_{cy})$ multiplied by the average number of such cycles $E(n)$

$$E(t_{sw}) = E(n) \cdot E(t_{cy}). \quad (112)$$

We then calculate the rate of switching $\gamma = 1/E(t_{sw})$ using $E(t_{cy})$ from Eq. (112) and $E(n)$ from Eq. (120) to obtain

$$\frac{1}{\gamma} = e^{k_b \cdot \tau} \cdot \left[ \left( \frac{1}{k_b} + \frac{1}{d_t} \right) - \frac{\tau}{e^{k_b \cdot \tau} - 1} \right]. \quad (122)$$

Equation (122) gives the switching rate from the uninduced state to the induced state $\gamma$ as a function of $\tau$. The terms $1/d_t$ and $1/k_b$ represent the average periods for which the lac-regulatory region is occupied and unoccupied by the repressor respectively ($t_b, t_u$ as defined previously in this section), while the $\frac{\tau}{e^{k_b \cdot \tau} - 1}$ term accounts for the fact that the operator region is never unoccupied for a period greater than $\tau$ until the switch occurs.

4.2 The threshold period

We now consider the dependence of the threshold period $\tau$ on external inducer concentration, thus connecting the switching rate $\gamma$ to external inducer concentration. The time interval $\tau$ needs to be long enough to produce enough pumps (LacY) such that positive feedback kicks in and the system switches to the induced state. As has been discussed in the main text and earlier in the SI, the LacY protein modulates its own production via two intermediaries – a sugar (TMG in this case) which is imported by LacY, and a repressor whose activity is reduced upon being bound by the sugar. The sugar thus acts as an inducer for LacY production.

Let the number of inducers inside the cell be $i_c$. The details regarding rates of inducer-repressor binding and unbinding are discussed in Section 2.5. For convenience, we define

$$\alpha = \frac{i_c s_b}{s_d}, \quad (123)$$

where $s_b$ is the rate at which an inducer binds to a repressor free of inducers and $s_d$ is the rate at which an inducer dissociates from a repressor with with all its inducer binding sites occupied as described in Section 2. If we
represent the fraction of repressors bound to \( j \) sugars by \( r_j \), at equilibrium we have

\[
\begin{align*}
\frac{r_1}{r_0} &= \alpha \\
\frac{r_2}{r_1} &= \frac{3}{8} \alpha \\
\frac{r_3}{r_2} &= \frac{\alpha}{6} \\
\frac{r_4}{r_3} &= \frac{\alpha}{16}.
\end{align*}
\] (124)

(125)

(126)

(127)

Since \( \sum_{j=0}^4 r_j = 1 \), we can calculate the fraction of repressor molecules which are free from inducers to be

\[
r_0 = \frac{1}{1 + \alpha + \frac{3}{8} \alpha^2 + \frac{\alpha^3}{16} + \frac{\alpha^4}{256}}.
\] (128)

If \( i_t \) is the number of internal inducers such that only about 10% of repressors are free of inducer (\( r_0 \approx 0.1 \)), using Eq. (128), we have \( i_t \approx 10000 \). The number of inducers inside the cell is determined by the rate of import by pumps (passive diffusion into the cell is a much weaker effect, see Section 2) acting against dilution (rate constant \( \alpha_{\phi} \)) and diffusion out of the cell (rate constant \( \sigma_{out} \)). Let \( Y_i \) be the number of LacY (pumps) needed to maintain \( i_t \) internal inducers. Denoting external inducer concentration in \( \mu M \) by \( i \) and using the function for sugar import defined in Section 2 we have

\[
i_t = \frac{\alpha_{tr} Y_i}{\alpha_{\phi} + \sigma_{out} i_0 + i}.
\] (129)

\[
Y_i = \frac{\alpha_{\phi} + \sigma_{out}}{\alpha_{tr}} \left( 1 + \frac{i_0}{i} \right).
\] (130)

At low external inducer concentrations more pumps are required to maintain a certain inducer level inside the cell. The threshold time period \( \tau \) is the time needed to make \( Y_i \) proteins if the operator remains free of repressors. Thus, \( \tau \) is related to \( Y_i \) via the rates of transcription (\( y_{tp} \)), translation (\( y_{tr} \)) and the mRNA lifetime (\( \alpha_{\phi m} \))

\[\tau = Y_i \frac{\alpha_{\phi m}}{y_{tp} y_{tr}}.\] (131)

Using Eq. (130) we obtain for the threshold period

\[
\tau = i_t \frac{\alpha_{\phi m}}{y_{tp} y_{tr}} \frac{\alpha_{\phi} + \sigma_{out}}{\alpha_{tr}} \left( 1 + \frac{i_0}{i} \right)
\] (132)

\[= \beta \left( 1 + \frac{i_0}{i} \right),\] (133)

with

\[
\beta = i_t \frac{\alpha_{\phi m}}{y_{tp} y_{tr}} \frac{\alpha_{\phi} + \sigma_{out}}{\alpha_{tr}}.
\] (134)

Substituting the values of the various parameters (from Section 2) along with the free parameter \( \alpha_{tr} = 9.44/min \) chosen to best fit the switching curve (as described in Section 2 and the main text), we obtain \( \beta = 0.16 \) minutes. In Fig. S5 we show how \( \tau \), \( Y_i \) and the number of unbinding-rebinding events before a switch depend on the external inducer concentration.
Figure S5: Figures (A,B,C) show how quantities critical to the switching process vary with external inducer concentration (X-axis). Figure (A) shows the threshold period $\tau$ as in Eq. (133) as a function of external inducer concentration, Figure (B) shows the number of pumps (LacY) that need to be produced to switch the system to the induced state (as in Eq. (130)) as a function of external inducer concentration while Figure (C) shows the external inducer concentration dependence of the average number of times the repressor-DNA loop is formed, broken (through total dissociation of the repressor from the lac-regulatory region) and re-formed (through a repressor binding its two binding sites on the lac-regulatory region) before a switch to the induced state takes place, see Eq. (120). Only at very high concentrations of TMG (greater than 200 µM) can a single unbinding event lead to a switch to the induced state as suggested in [5]. However, at low concentrations of TMG many thousands of binding-unbinding-rebinding cycles can happen before the cell switches to the induced state. At intermediate and low concentrations of TMG we find that a few hundred LacY molecules are needed for positive feedback to kick in and the cell to switch, consistent with the experiments of Choi et al. described in [5]. At 20 µM TMG, around 400 LacY molecules need to be produced and the gene must remain unoccupied by repressors for approximately 2 minutes. The DNA-repressor loop will be broken and reformed about 40 times before the switch happens, corresponding to a switching rate of $7.88 \times 10^{-4}/\text{min}$. 
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