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## Supplemental Information

# Simulating Genetic Circuits in Bacterial Populations with Growth Heterogeneity

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**Simulation algorithm**

All simulations are run with different versions of Gillespie's stochastic simulation algorithm [1, 2]. In these simulations, the dynamics of the various reaction channels are characterized by their propensities $a_j$. These propensities determine the probabilities of the time $\tau$ when the next reaction will take place, given by the probability density $p(\tau) = a\ \exp(-a\tau)$, where $a = \sum_j a_j$ is the sum of propensities, and which of the reactions is chosen, determined by the probabilities $p(j) = a_j/a$. In the Direct Method (which we use here for comparison) we draw two random numbers $r_1$ and $r_2$ from a uniform distribution on the unit interval and calculate the time of the next reaction as $\tau = t + \frac{1}{a}\ln(\frac{1}{r_1})$ and the index of the next reaction as $j =$ the smallest integer satisfying $\sum_{j'=1}^{j} a_{j'}/a > r_2$. With this we proceed the time of the simulation to $t = \tau$, carry out the reaction $j$, record the desired quantities and calculate new set of propensities for the next reaction.

In the Next Family Method we group the reactions into families; each family consists of the reactions of one cell. These families are then considered as pseudo-reactions with propensities $a^f = \sum_{j=1}^{l^f} a_j^f$, where $l^f$ is the number of reactions in the family/cell $f$. Next, we generate the expected times of the next reaction for each family as $\tau^f = t + \frac{1}{a^f}\ln(\frac{1}{r_1})$ and heapify these so that they are in a tree-ordered data structure, in which the node representing the family (say $m$) is the one with the smallest time $\tau^m$ [3]. This gives us the time of the next reaction in the population as $t = \tau^m$ and the cell in which the reaction is happening as $m$. To obtain the actual reaction that is firing we apply the Direct Method to the $l^m$ reactions in this family. With this we then proceed the time of the simulation to $t = \tau^m$, implement that reaction, sample the desired quantities, calculate the new set of propensities ($a_{new}^m$) and repair the heap starting from this nodal position based on its new expected time for the next reaction, $\tau_{new}^m = t + \frac{1}{a_{new}^m}\ln(\frac{1}{r_1})$. If no other cell is affected by this reaction, we do not have to do any other heap repair step and can read off the time and index of the next reaction in the population from the updated heap in similar way. For the Chemostat, if the next reaction happens to be the production of a daughter cell, we produce a copy of the dividing cell and replace a randomly chosen cell (say $r_3$) with it. In this case the heap has to be repaired starting at the position of the cell $r_3$. To obtain this position we use indexed heap data structure so that if the heap consists of ordered pairs $(f, \tau^f)$, the

$f$-th element of the index structure will point to the position of the $(f, \tau^f)$ pair in the heap.

**Mutual repressor system with growth inhibition**

In a simple Mutual Repressor the deterministic equations for the evolution of the concentrations of the two proteins $p_i$ are given by

$$\begin{aligned} \frac{dp_1}{dt} &= \alpha_1 \frac{1}{1+(p_2/K_2)^{n_2}} - (\beta_{01}+\lambda)p_1 \\ \frac{dp_2}{dt} &= \alpha_2 \frac{1}{1+(p_1/K_1)^{n_1}} - (\beta_{02}+\lambda)p_2 \end{aligned} \tag{1}$$

where $\alpha_i$ are the synthesis rates of the proteins, $\beta_{0i}$ are the degradation rates due to proteolysis and $\lambda$ is the growth rate, related to cell division time $T_d$ as $\lambda = \ln 2/T_d$. Note that here we have described the two step protein synthesis process, involving transcription and translation, as an effective one step process. Moreover, it is an implicit cell division model where we include the dilution rate due to cell growth and division into the degradation term, i.e., we study the effective average behavior in the cell at times longer than the cell division time. $K_i$ are the threshold concentrations for repression and $n_i$, the Hill coefficients, represent the cooperativity of binding needed for repression, both being determined by the architecture of the corresponding promoter regions.

The above system is known to exhibit bistability for $n_i \geq 2$. To incorporate a difference in growth rates between the two states, we assume the protein $P_1$ to be inhibitory to the growth of the cell. We model this toxicity to growth as

$$\lambda = \lambda_{min} + \frac{\Delta\lambda}{(1+p_1/K_\lambda)}, \tag{2}$$

where $\Delta\lambda = (\lambda_{max} - \lambda_{min})$ and $K_\lambda$ is the characteristic concentration for the protein to be toxic. To study phenotype switching, we treat the above set of equations as a stochastic chemical reaction system and simulate it with the Stochastic Simulation Algorithm described above. The propensities for this system are given as $a_1 = \alpha_1 \frac{1}{1+(p_2/K_2)^{n_2}}$, $a_2 = \alpha_2 \frac{1}{1+(p_1/K_1)^{n_1}}$, $a_3 = (\beta_{01}+\lambda)p_1$ and $a_4 = (\beta_{02}+\lambda)p_2$. $\lambda$ is instantaneously determined by Eq. 2. The parameters used are $\alpha_1 = \alpha_2 = 3\ \mu M\ \text{min}^{-1}, \beta_{01} = \beta_{02} = 0.005\ \text{min}^{-1}, K_i = K_\lambda = 20\ \mu M, \lambda_{max} = 0.04\ \text{min}^{-1}$. $\lambda_{min}$ is varied to modulate the toxicity.

In the more realistic case, the synthesis rate is taken to depend on the growth rate of the

cell using the expression from ref. [4]

$$\frac{dp_1}{dt} = \frac{\alpha_1[1-\exp(-\lambda/\lambda')]}{1+(p_2/K_2)^{n_2}} - \beta_1 p_1$$
$$\frac{dp_2}{dt} = \frac{\alpha_2[1-\exp(-\lambda/\lambda')]}{1+(p_1/K_1)^{n_1}} - \beta_2 p_2. \quad (3)$$

The toxicity is described by

$$\lambda = \frac{\lambda_{max}}{(1+p_1/K_\lambda)}. \quad (4)$$

and varied by modulating the threshold concentration $K_\lambda$. The parameter values are given in the caption of Fig. S6.

**Bistable antibiotic resistance circuit**

The bistable resistance circuit described in Ref. [5] is based on a positive feedback in which expression of a resistance gene reduces the intracellular antibiotic concentration. The latter reduction speeds up growth, which has a positive effect on expression of the resistance gene, because growth in translation-limited conditions increases the expression of constitutively expressed genes [6] such as the resistance gene. The three components of that positive feedback are described as follows: The growth rate of the cell in the presence of antibiotic is given by

$$\lambda = \frac{\lambda_0}{(1+[\mathrm{Cm}]_{\mathrm{int}}/I_{50})},$$

where $\lambda_0$ is the growth rate in the absence of intracellular antibiotic and $I_{50}$ is the concentration of intracellular antibiotic that reduces the growth rate by half. $[\mathrm{Cm}]_{\mathrm{int}}$ is the concentration of the antibiotic inside the cell, which is obtained from the extracellular concentration by balancing its diffusion in and out of the cell with its degradation or deactivation by the resistance protein CAT. This balance is given by

$$\kappa([\mathrm{Cm}]_{\mathrm{ext}} - [\mathrm{Cm}]_{\mathrm{int}}) = \frac{k_c p}{1+K_m/[\mathrm{Cm}]_{\mathrm{int}}}$$
$$\text{or, } [\mathrm{Cm}]_{\mathrm{int}} = \frac{1}{2}\left[[\mathrm{Cm}]_{\mathrm{ext}} - (K_m + \frac{k_c}{\kappa}p) + \sqrt{(K_m + \frac{k_c}{\kappa}p - [\mathrm{Cm}]_{\mathrm{ext}})^2 + 4K_m[\mathrm{Cm}]_{\mathrm{ext}}}\right],$$

where $\kappa$ is the permeability of the cell membrane to Cm, $K_m$ is the affinity of CAT to Cm, $k_c$ the maximal rate of turnover of Cm by CAT , and $p$ is the concentration of CAT.

The expression levels of unregulated proteins in the cell depend linearly on the growth rate of the cell under sub-inhibitory concentrations of translation-inhibiting antibiotics, so that

$$p = \frac{p_0}{\lambda_0}\lambda,$$

where quantities with suffix 0 indicate values in the absence of the antibiotic. This dependence is included in the model by choosing the synthesis rates as a quadratic function of the growth rate. Similar to the model of the toggle switch, we formulate a deterministic rate equation as

$$\frac{dp}{dt} = \alpha\lambda^2 - (\beta_0 + \lambda)p,$$

with $\alpha = p_0/\lambda_0$, so that now the propensities for the stochastic simulation are $a_1 = \alpha\lambda^2$ and $a_2 = (\beta_0 + \lambda)p$. We simulate the above set of equations in the same way as for the previous model, with $\lambda$ and $[\mathrm{Cm}]_{\mathrm{int}}$ taken as instantaneous quantities. The model parameters are obtained from Table S2 of Ref. [5] as $\lambda_0 = 0.01$ min$^{-1}$, $I_{50} = 5.5\ \mu M$, $K_m = 12\ \mu M$, $k_c = 36000$ min$^{-1}$, $p_0 = 14.2\ \mu M$ for the strain Cat1; $\beta_0$ was set to a small non-zero value, $\beta_0 = 0.0002$ min$^{-1}$ and $\kappa$ was taken to be 126 min$^{-1}$ to match the MIC in the Chemostat simulation to the experimentally observed value.


[1] Gillespie, D. T. (1976). A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. J. Comput. Phys. 22, 403-434.

[2] Gillespie, D. T. (1977). Exact stochastic simulation of coupled chemical reactions. J. Phys. Chem. 81, 2340-2361.

[3] Sedgewick, R. (1998). Algorithms in C. Addison-Wesley, Reading.

[4] Hintsche, M., Klumpp, S. (2013). Dilution and the theoretical description of growth-rate dependent gene expression. J. Biol. Eng. 7, 22.

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[6] Scott, M., Gunderson, C. W., Mateescu, E. M., Zhang, Z., Hwa, T. (2010) Inter-dependence of cell growth and gene expression: Origins and consequences. Science 330, 1099-1102.

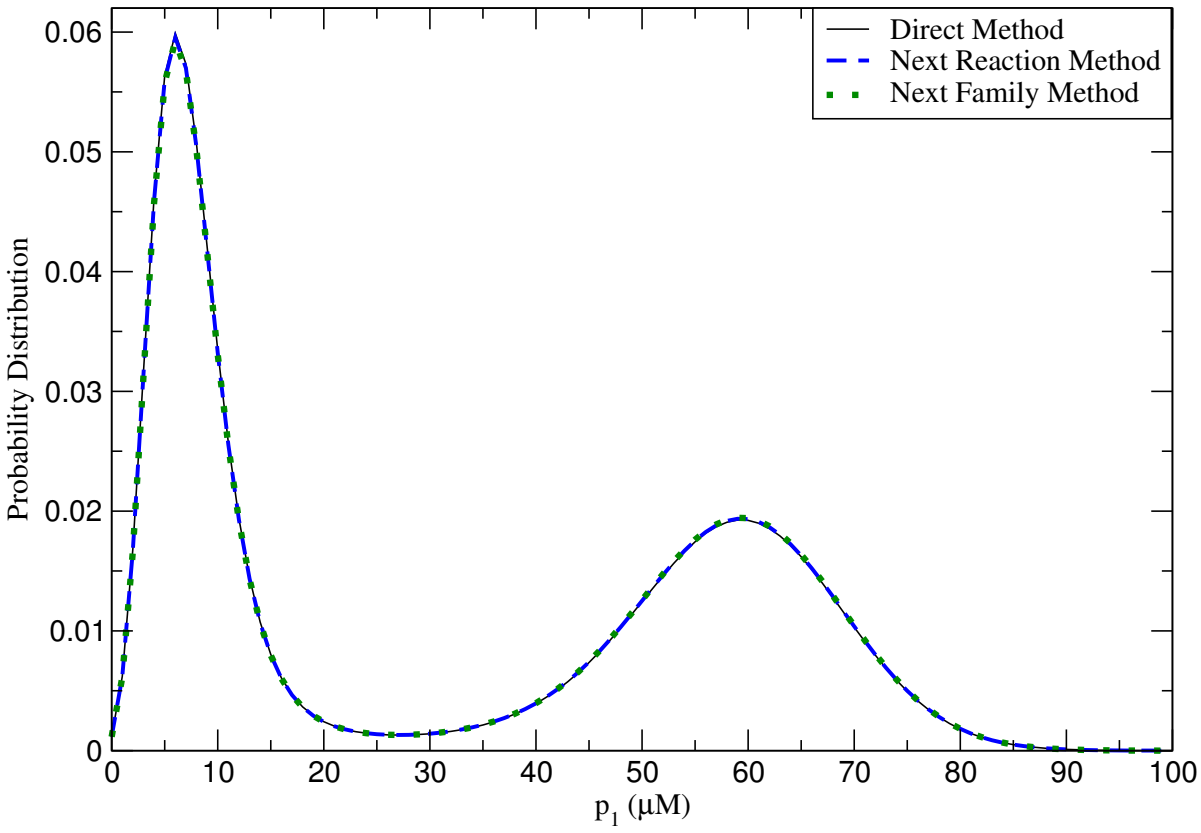


FIG. S1. Distribution of protein concentration obtained from the three methods are statistically identical. Here, the sampling is done over $10^6$ steps of 5 min each after 1000 min of equilibration (i.e. for 5,001,000 min) and over 1000 cells.

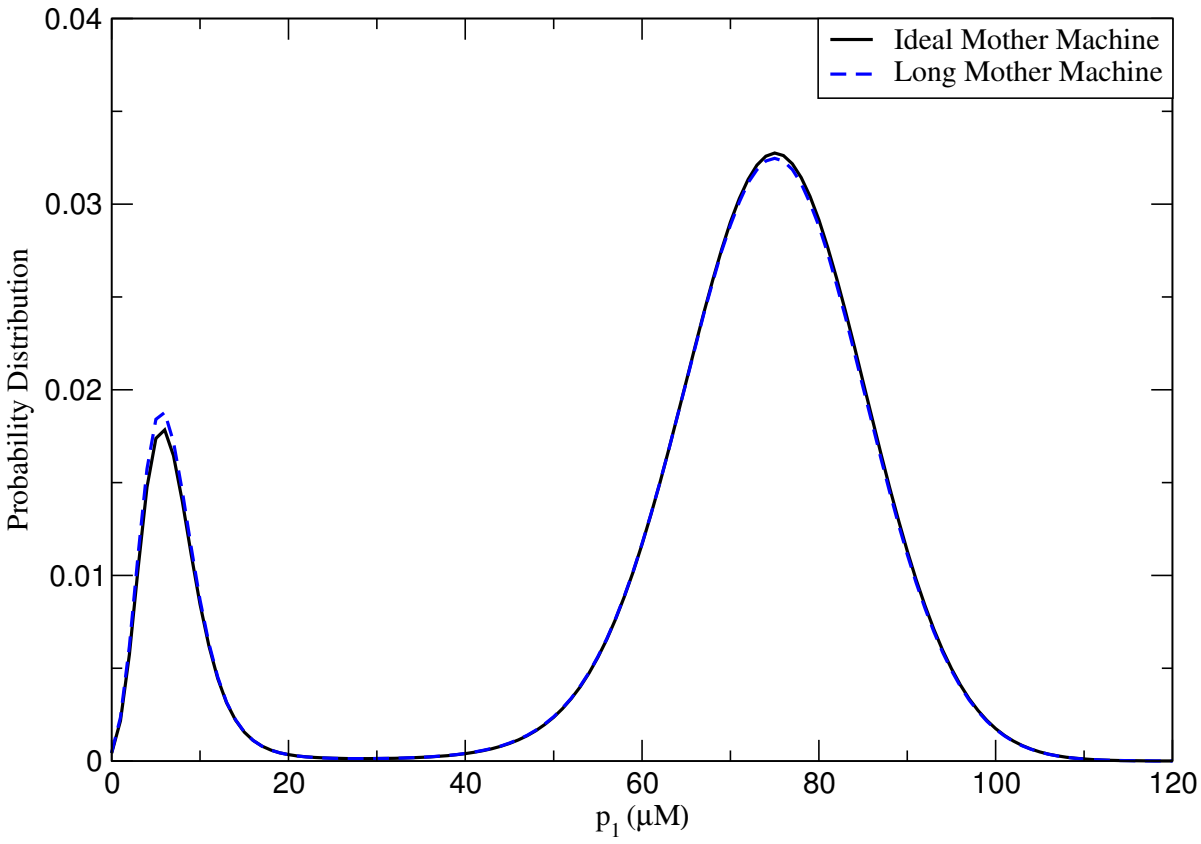


FIG. S2. Distribution of protein concentration obtained in the ideal Mother Machine is qualitatively similar to that in a 20 cell long channel Mother Machine. Here the results are for the case where $P_1$ is toxic so that high $p_1$ state is more probable.

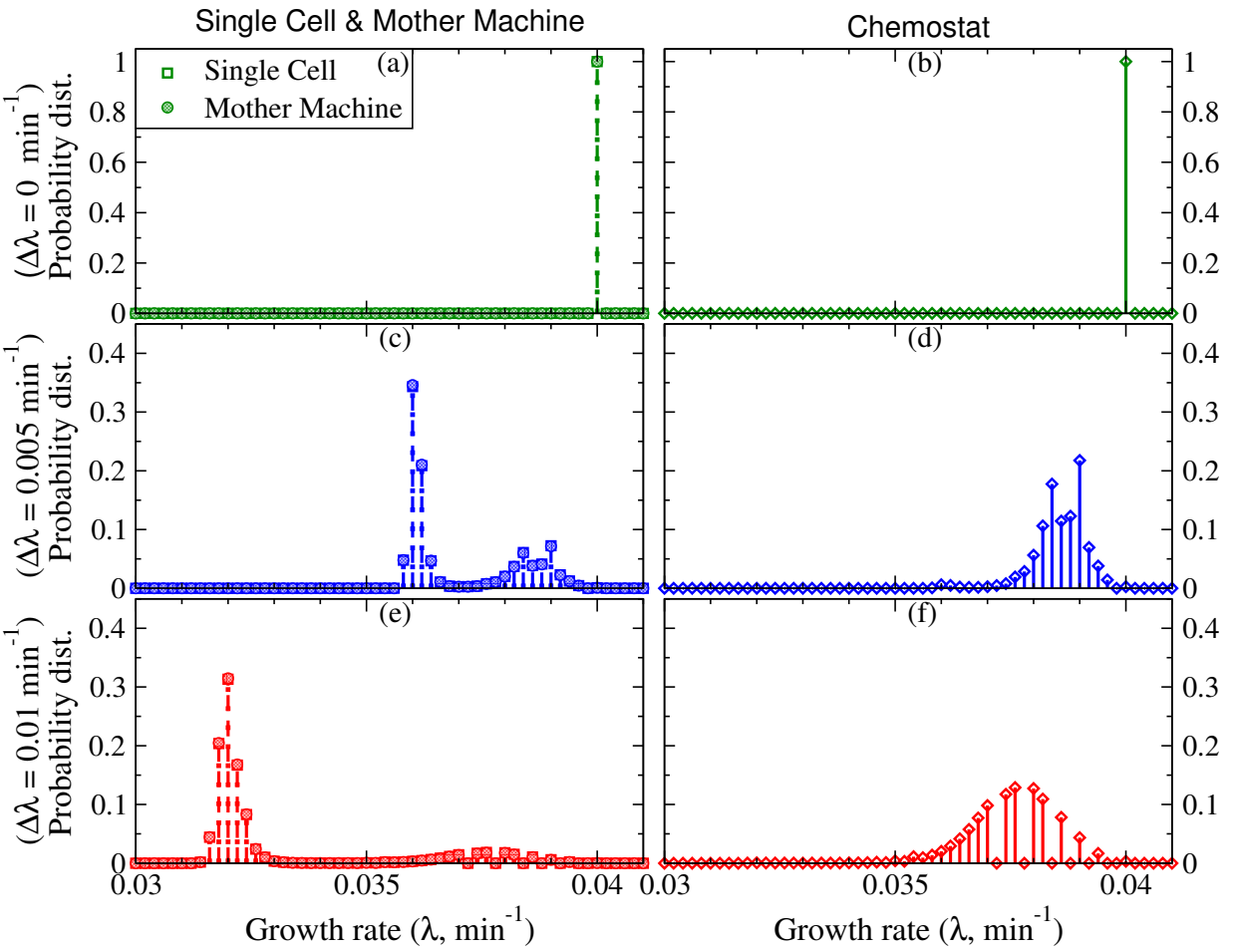


FIG. S3. Distribution of growth rates for the toxic toggle switch in a single cell (squares with dashed dropdown lines) and in the two population setups (shaded circles with dotted dropdown lines for the Mother Machine and diamonds with solid dropdown lines for the Chemostat). The parameters are the same as in Fig. 4. A bin of 0.002 $\mathrm{min}^{-1}$ has been used.

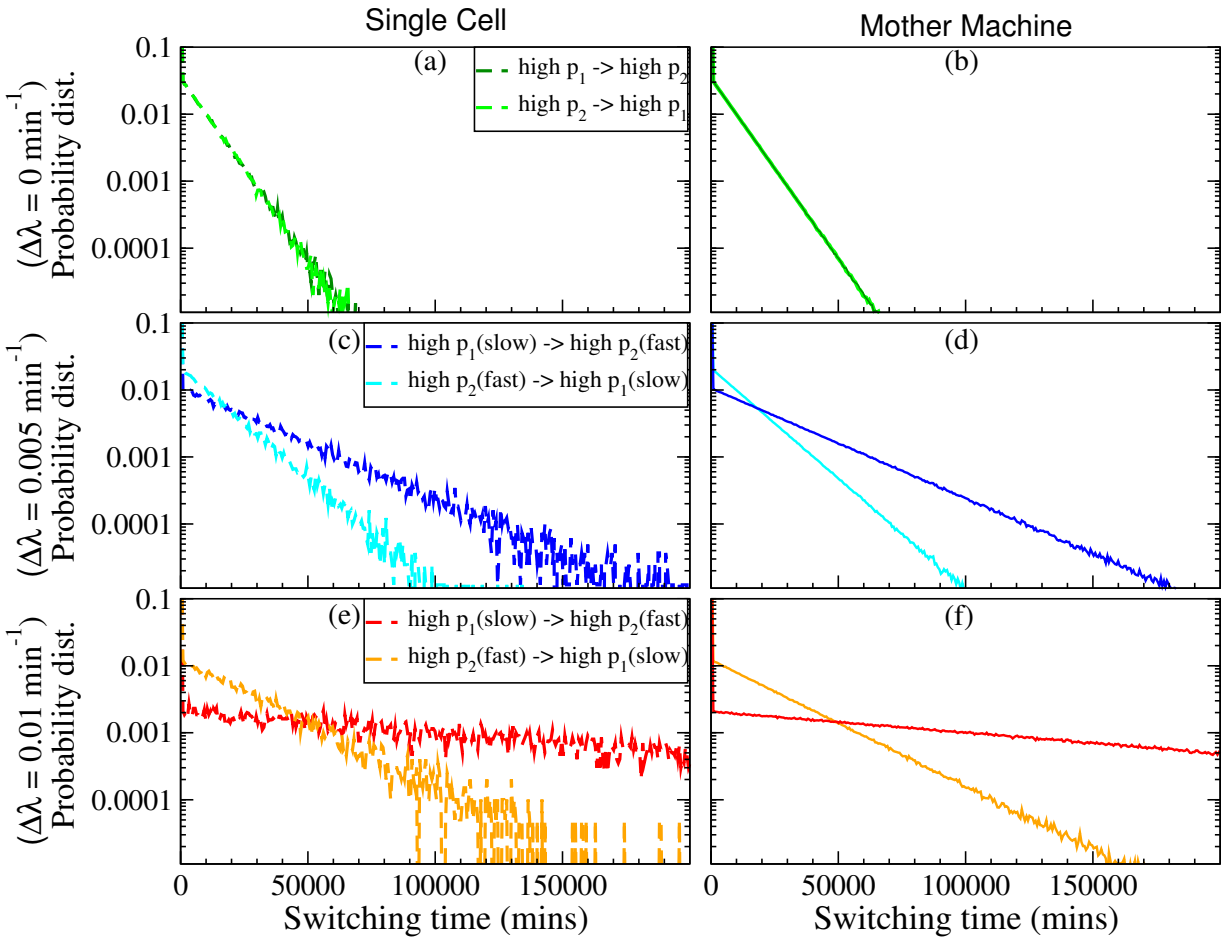


FIG. S4. Distribution of phenotype switching times for the toxic toggle switch: switching times from slow growing to fast growing (dark green, blue, red) and from fast growing to slow growing (light green, cyan, orange) states, for a single cell (dashed curves) and for the Mother Machine population (solid curves). The sampling is done over $10^8$ time steps of 15 min each for the single cell, and over 1000 cells and $10^7$ time steps of 15 min each for the Mother Machine population. A bin of 600 min has been used.

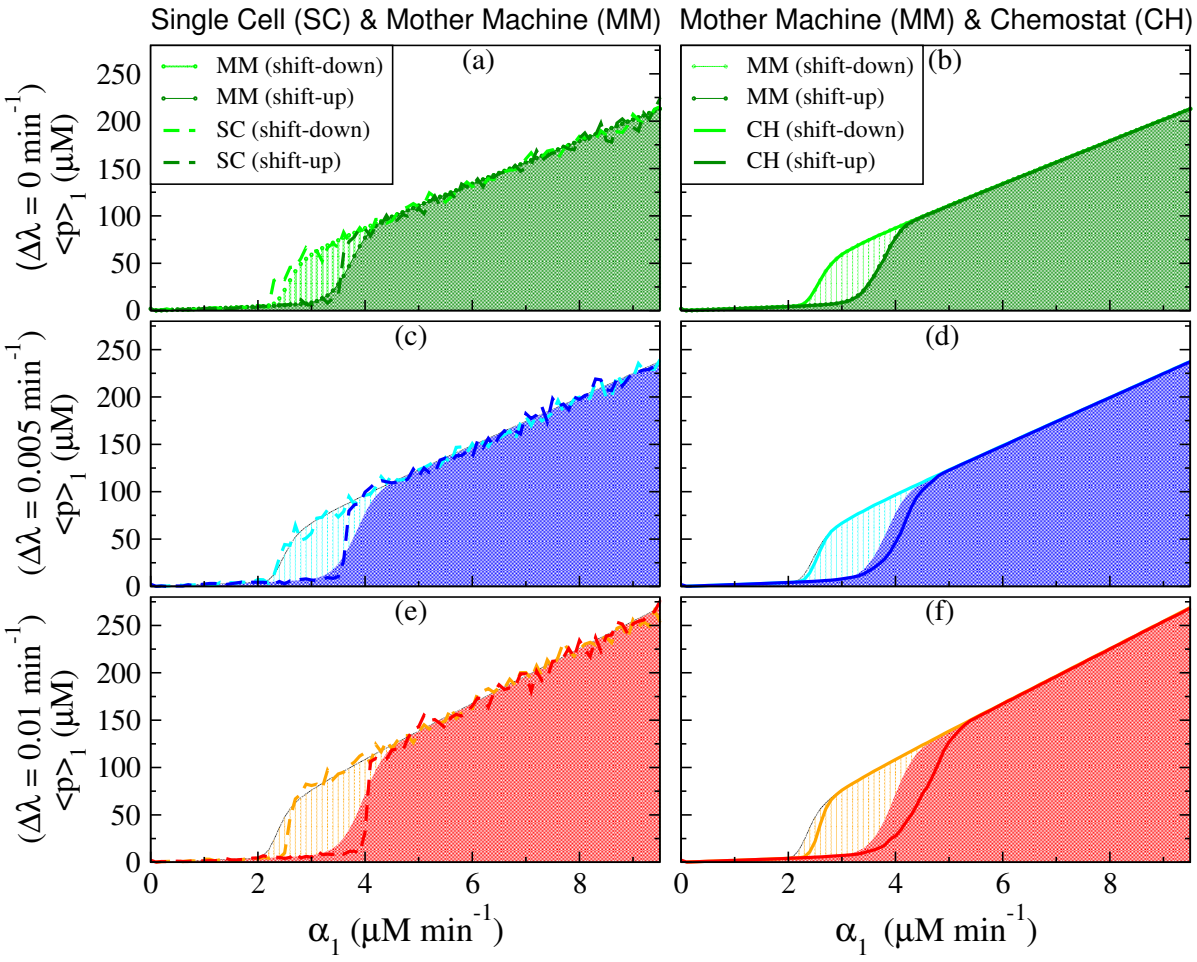


FIG. S5. Hysteresis curves for the toxic toggle switch: Hysteresis plots for the concentration of protein $P_1$ as its synthesis rate is varied (dark green, blue, red for shift up of the synthesis rate and light green, cyan, orange for shift down). The dashed curves are for the single cell, the shaded down curves are for the Mother Machine and the solid curves are for the Chemostat. The averaging is done for 100 steps of 2 min each for the Single cell. For the two populations a further averaging over 10000 cells has been done. We note that the noise in the single cell plots is because of averaging over a small time. Averaging over times longer than switching times will collapse the hysteresis curves. This further highlights the advantage of having the possibility to average over many cells in the Mother Machine, even for studying single cell statistics.

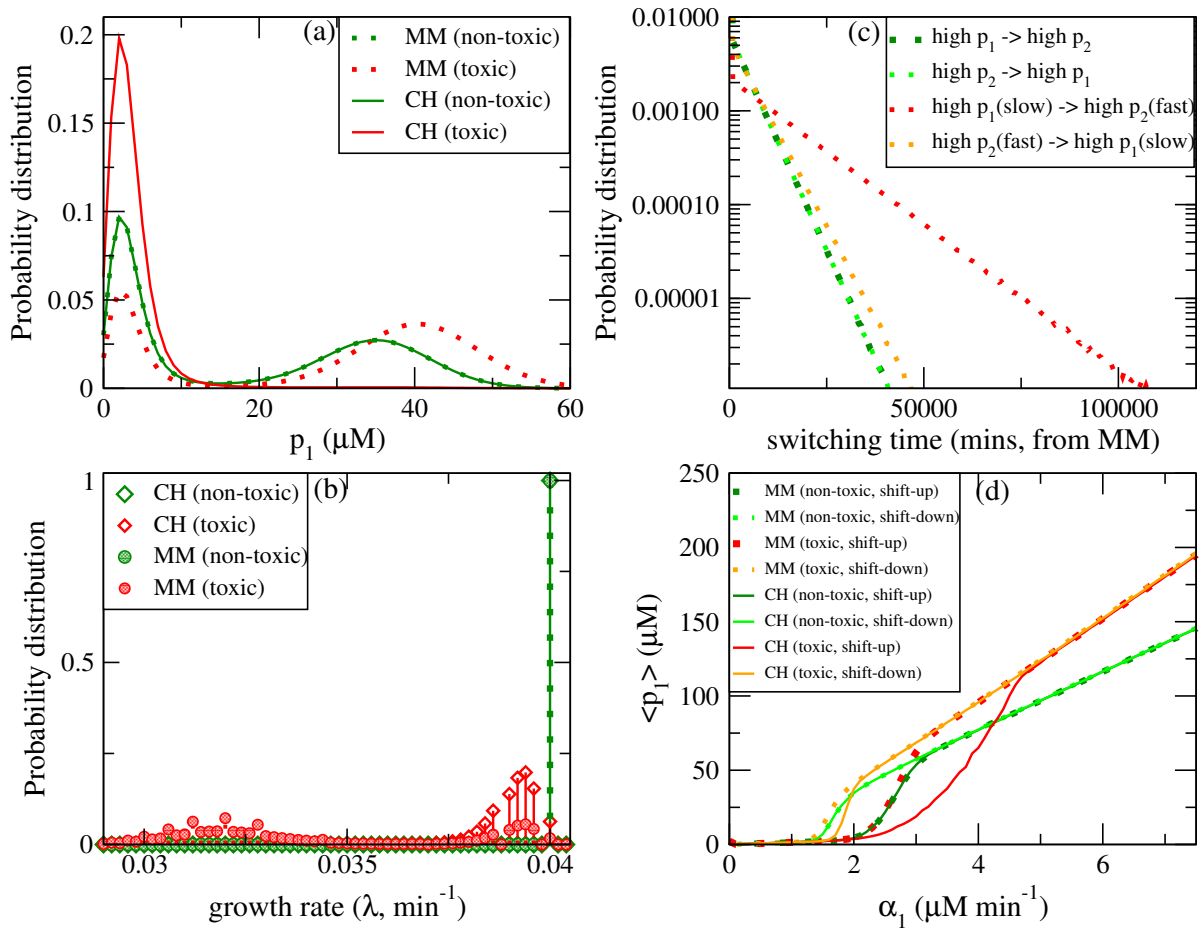


FIG. S6. Results for a more realistic model of toxic toggle switch: The toggle switch model with growth inhibition by one of the two proteins ($P_1$) is extended to include a growth-rate dependence of the synthesis rates; growth inhibition is modulated by varying the threshold concentration in a repression function. Distributions of (a) the concentration of the protein $P_1$, (b) of the growth rate (green curves are without growth inhibition (infinite $K_\lambda$, non-toxic), red curves are with growth inhibition ($K_\lambda = 160\ \mu M$, toxic)) and (c) of the switching times from slow growing to fast (dark green, red) and from fast growing to slow (light green, orange). (d) Hysteresis curves upon variation of the synthesis rate of protein $P_1$ (dark green and red curves for shift-up and light green and orange for shift-down). The parameters are $\alpha_1 = \alpha_2 = 2\ \mu M\ \text{min}^{-1}, \beta_{01} = \beta_{02} = 0.005\ \text{min}^{-1}, K_1 = K_2 = 10\ \mu M, \lambda_{max} = 0.04\ \text{min}^{-1}, \lambda' = 0.02\ \text{min}^{-1}$. Dotted curves are for the Mother Machine population, while the solid curves are for the Chemostat population. Parameters for averaging and binning are the same as in the corresponding figures of the simple toxic toggle switch.

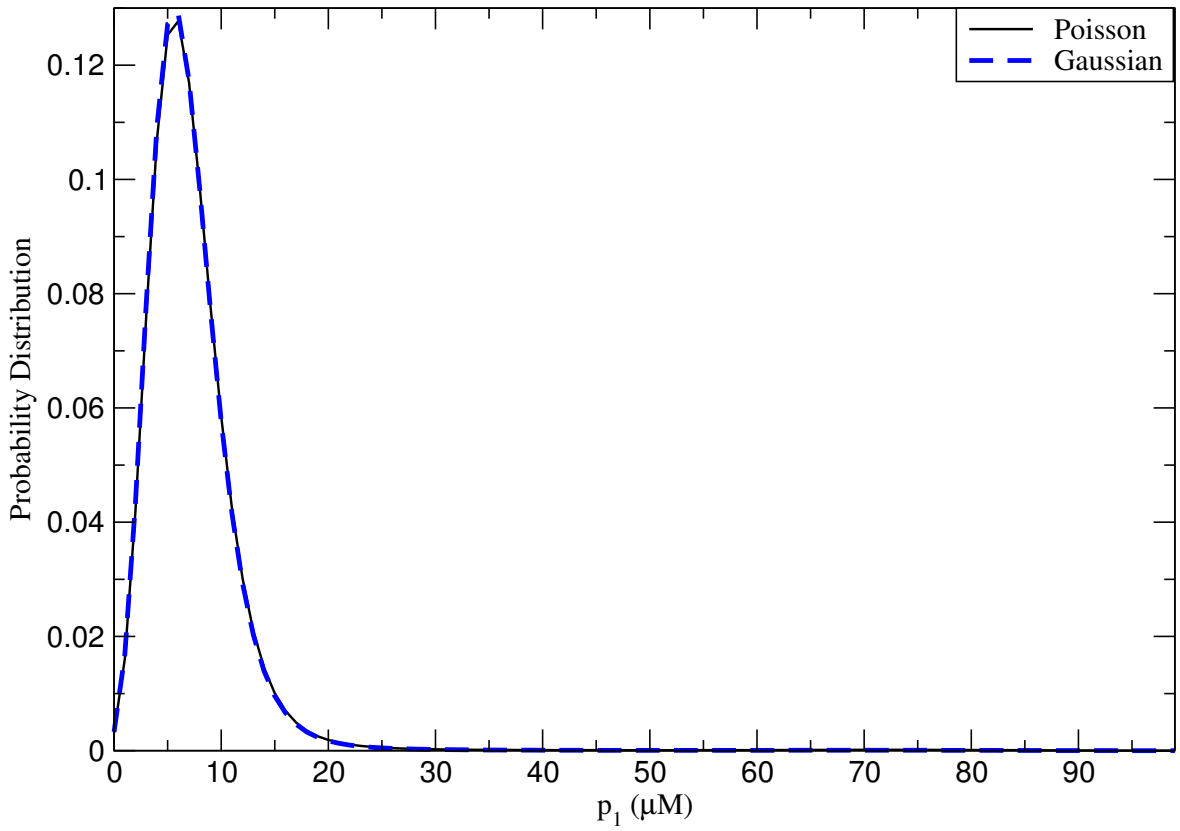


FIG. S7. Distribution of protein concentration obtained by selecting division times from an uncorrelated Gaussian distribution is qualitatively similar to that obtained by selecting from an uncorrelated exponential distribution as in Poisson processes. The result is for the simple toxic toggle switch with $\Delta\lambda = 0.01$ min$^{-1}$.

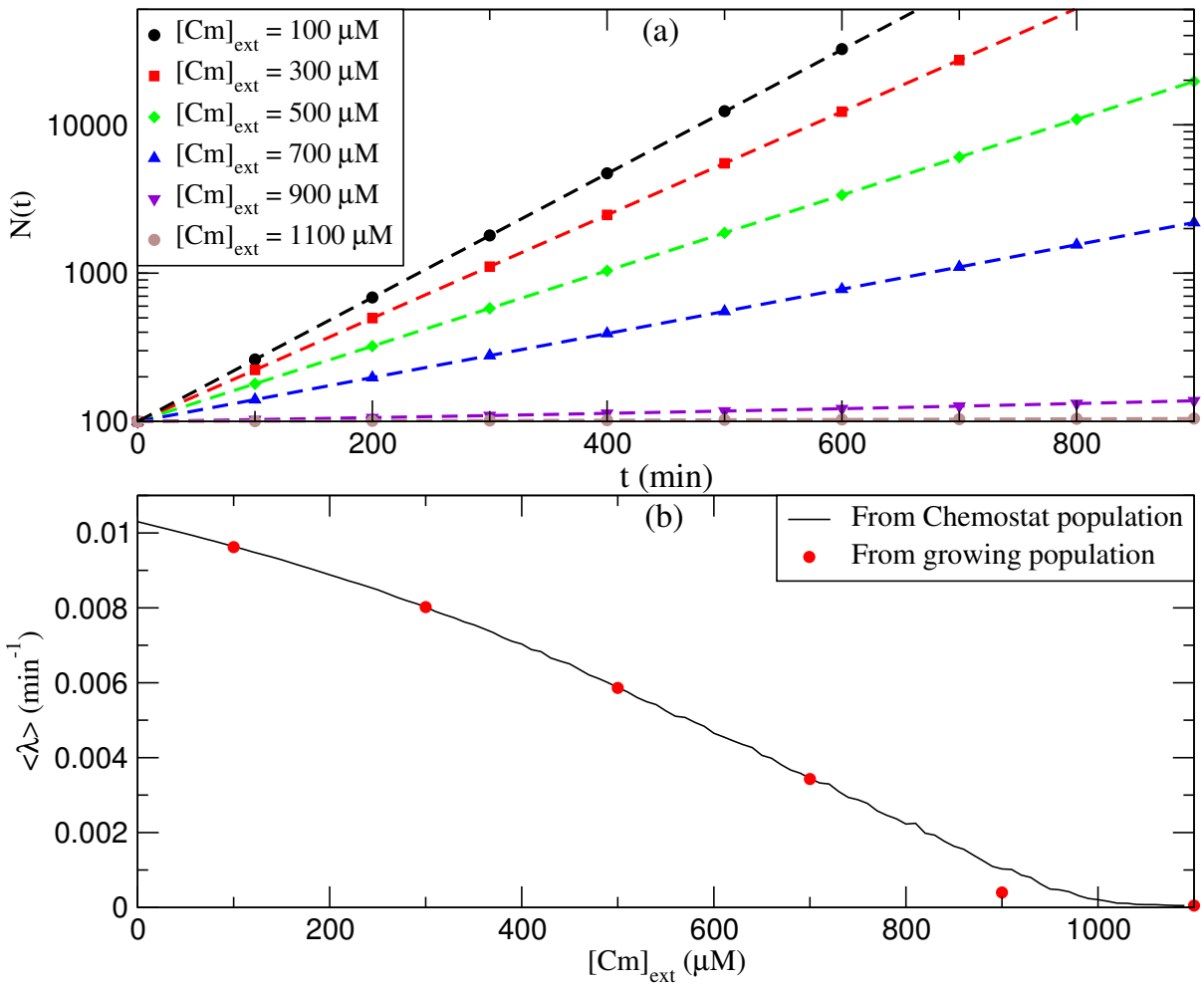


FIG. S8. Growth curve obtained from a growing population gives similar result to that obatined from the Chemostat population : (a) The system is equilibrated at different antibiotic concentrations and then grown for 900 min, starting from 100 cells. The result plotted is average over 1000 such realizations. (b) The average growth rates at each antibiotic concentrations is obtained by fitting an exponential to the curves in (a).