Entraining synthetic genetic oscillators

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We propose a new approach for synchronizing a population of synthetic genetic oscillators, which consists in the entrainment of a colony of repressilators by external modulation. We present a model where the repressilator dynamics is affected by periodic changes in temperature. We introduce an additional plasmid in the bacteria in order to correlate the temperature variations with the enhancement of the transcription rate of a certain gene. This can be done by introducing a promoter that is related to the heat shock response. This way, the expression of that gene results in a protein that enhances the overall oscillations. Numerical results show coherent oscillations of the population for a certain range of the external frequency, which is in turn related to the natural oscillation frequency of the modified repressilator. Finally we study the transient times related with the loss of synchronization and we discuss possible applications in biotechnology of large-scale production coupled to synchronization events induced by heat shock. © *2009 American Institute of Physics*.
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The development of synthetic genetic networks has focused the attention of many scientists during the past decade.[1] Their relevance is due to the fact that simple synthetic genetic networks can help us in the comprehension of their natural counterparts. Moreover, synthetic biology can usefully improve bioprocesses production by introducing alternative cellular designs. For example, biopharmaceutical manufacturing is nowadays moving toward metabolic engineering.[2,3] Furthermore, synthetic gene networks would provide the basis for highly sophisticated genetic manipulations in mammalian cells leading to biopharmaceutical manufacturing, gene therapy, and tissue engineering applications. The genetic oscillator known as the repressilator[4] has become a paradigmatic example of how to design a genetic network with specific dynamics. Despite that the repressilator shows oscillations of its protein concentrations, the frequency and phase heterogeneity that exist from cell to cell destroy the eventual coherent behavior in a colony of repressilators. In the current work we propose a modification of this genetic oscillator in order to externally control the oscillatory dynamics of a population of repressilators. The numerical results presented in this paper give some hints about how to induce global oscillations at the protein level of the colony and how the transient time to the synchronization/desynchronization strongly depends on the stochastic nature of the system. Concerning the global oscillations, it is of particular interest to study the repressilator in order to develop a mammalian clock as a model for natural circadian clock.[5]

I. INTRODUCTION

Cells are complex systems which require the establishment of a high number of interactions between their multiple types of components. The cross regulation among protein factors and gene expression is an example. The understanding of these interactions is crucial since they regulate the essential cellular processes. Despite that the molecular bases of these processes are already known, the behavior of gene regulatory networks still remains poorly understood due to the complexity of their components as well as their multiple specific interactions. Recently, a novel approach appeared to study the interactions between genes and proteins.[1] It is based on the design of synthetic genetic networks, much simpler than the physiological ones operating within the cells. Due to their simplicity, the synthetic genetic networks can be constructed in the laboratory, and mathematical models can be developed in order to proceed to their numerical analysis. Two seminal papers boosted the study of such networks by designing and constructing two types of synthetic genetic networks. One of them is named repressilator,[4] and the other one is the genetic *toggle switch*.[6] In the present work we focus on the study of the repressilator, the genetic network proposed by Elowitz and Leibler,[4] which consists of three genes repressing each other in a closed chain. The repressilator is able to induce oscillations in the intracellular levels of three proteins encoded by the sequence of a plasmid, which is hosted by the unicellular bacterial microorganism *Escherichia coli* (*E. coli*).[4] In spite that oscillations were reported in different cell types and they were maintained even after cell division, it was also shown that the whole cell colonies evolved with different phases and frequencies. In

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order to overcome this lack of synchrony, a quorum sensing mechanism[7] was proposed to allow cell-to-cell communication, i.e., coupling between dynamical units. The model reported by García-Ojalvo *et al.*[8] showed that a modification of the repressilator plasmid, by adding the quorum sensing mechanism, led to the synchronization of the whole colony, even when moderate stochastic processes were considered. More recently, it has been shown in Ref. 9 that oscillations of the colony can be controlled by periodic injection of a chemical in the medium. In this paper we describe a feasible parallel technique, which consists in the entrainment of a colony of repressilators by using periodic increases in temperature as external perturbation. In our model, an additional plasmid is included in *E. coli* in order to direct the heat-shock mechanism of the cell, activated by an increase in temperature, to the production of one of the three oscillating proteins of the original repressilator as a target protein. Its expression levels will determine the frequencies of the other two, and under the adequate conditions, it will be possible to entrain oscillations of the whole colony. This way, we do not change directly the genetic load of the repressilator plasmid, in contrast with the modification proposed in Ref. 8. Numerical data reveal the appearance of regions of entrainment when the frequency of the temperature shocks is varied. Thus, we do not only show that synchronized oscillations of the whole colony can be accomplished, but we also find that only some external frequencies allow synchronization. Finally, we analyze the transition from the synchronized to the unsynchronized state and we show how cells loose synchrony when the external signal is suppressed.

II. REDESIGNING THE REPRESSILATOR

In this report we describe a method for entraining a population of repressilators, which actually represents a modification of the original repressilator. In our method we act on the environment of the cells in such a way that an external stimulus influences the cycle of the repressilator, instead of relying on cell to cell communication, as proposed in Refs. 8 and 10. In its original design, the repressilator consists of two plasmids. One of them is a plasmid containing three in-chain repressor genes in such a way that levels of encoded proteins oscillate, as it is shown by monitorization of variations in the expression of the reporter gene, encoding the green fluorescence protein (GFP), which is localized in the second plasmid. Since the GFP sequence is coupled to a promoter corresponding to one of the proteins in the repressilator, if this protein is produced it will repress the production of the GFP. In this way, it is possible to detect the oscillations of the system by measuring the fluorescence emitted by the cell. The reporter plasmid could be modified in order to obtain a third plasmid containing a promoter sensitive to an external stimulus (Fig. 1). Along with the promoter, the gene encoding for one of the (already existing) repressilator proteins is added. As the external perturbations are applied, the proteins dependent on the stimulus-sensitive promoter should be produced. The extra amounts of one of the three repressilator proteins influence the cycle of the network, and if all cells are submitted to the same external stimulus, entrainment of the whole population could be obtained. The method proposed here for the external control is to modify periodically the temperature of the colony, increasing its value to 42 °C during controlled time intervals. The heat-shock response pathway of the *E. coli* includes several chaperone proteins, which are essential for the survival of the bacteria as the temperature increases. Obtaining these proteins depends on the related gene expression from a promoter, which is recognized by specific bacterial protein factors that allow RNA polymerases to start gene transcription.[11] We choose the groE promoter, which is coupled artificially to the lacI gene. In this way we can control externally the level of LacI proteins and, in turn, the repressilator. The groE promoter is recognized by a transcription complex which includes, among other proteins, the RNA polymerase and a protein σ^{32} that is only produced when the heat-shock mechanism is turned on.[11] Controlled by the groE promoter, this machinery expresses the gene and obtains LacI proteins only when the groE promoter is recognized. Since LacI is one of the three repressor proteins in the repressilator, an excess of LacI synthesis (when heat-shock mechanism is activated) will affect the oscillations of the three protein levels.

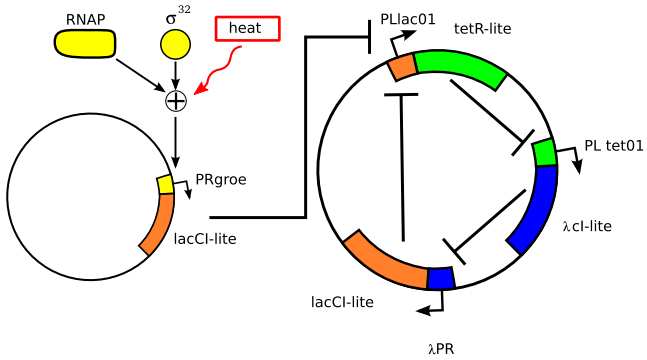

FIG. 1. (Color online) Schematic representation of the repressilator (right) and the additional plasmid (left). Reporter plasmid is not represented. The plasmid on the right contains three repressor genes, which are repressing one to each other in a closed chain. The promoters of each gene are repressed by three proteins (CI, LacI, and TetR), which are in turn encoded by each one of the three genes. The plasmid on the left contains a heat-shock sensitive promoter (PRgroE) controlling the lacI gene expression. When the temperature increases, the heat-shock response is activated and the transcription process of the LacI protein begins.

The mathematical model of the repressilator was proposed in Ref. 4 and it is based on three pairs of ordinary differential equations, one pair for each gene, describing the evolution of the mRNA (m_i) and the corresponding protein number (p_i),

$$\frac{dm_1}{dt} = -\gamma_{m1} m_1 + \frac{\alpha_1}{1 + (p_3/K_0)^n}, \tag{1}$$

$$\frac{dp_1}{dt} = a_1 m_1 - \gamma_{p1} p_1, \tag{2}$$

$$\frac{dm_2}{dt} = -\gamma_{m2} m_2 + \frac{\alpha_2}{1 + (p_1/K_0)^n}, \tag{3}$$

$$\frac{dp_2}{dt} = a_2 m_2 - \gamma_{p2} p_2, \tag{4}$$

$$\frac{dm_3}{dt} = -\gamma_{m3} m_3 + \frac{\alpha_3}{1 + (p_2/K_0)^n}, \tag{5}$$

$$\frac{dp_3}{dt} = a_3(m_3 + m_4) - \gamma_{p3} p_3. \tag{6}$$

Subindex $i=1,2,3$ corresponds to genes tetR, cI, and lacI, respectively. Parameters γ_{pi} and γ_{mi} are the half-life time of proteins and mRNA, α_i is the transcription rate of mRNA, and a_i accounts for the number of translated proteins per mRNA molecule. Note that the evolution of the LacI protein in Eq. (6) is affected by m_4, which corresponds to an additional mRNA transcription derived from a source not localized in the repressilator. As explained above, this source consists of an extra plasmid that produces an excess of mRNA that will be translated into LacI protein, only in the presence of σ^{32}. The equations corresponding to the activation of the promoter groE by heat shock, which results in the production of σ^{32} and m_4, are

$$\frac{d\sigma^{32}}{dt} = -\gamma_{32}\sigma^{32} + k_{32} f_T(t), \tag{7}$$

$$\frac{m_4}{dt} = a_{32}\sigma^{32} - \gamma_{m_4} m_4. \tag{8}$$

The concentration of σ^{32} is controlled by the temperature of the system, which is externally modulated by the function $f_T(t)$. In this case $f_T(t)$ is a stepwise function given by

$$f_T(t) = \begin{cases} 1 & \text{when } kT_{\text{ext}} \le t < kT_{\text{ext}} + T_d, \\ 0 & \text{when } kT_{\text{ext}} + T_d \le t < (k+1)T_{\text{ext}}, \end{cases} \tag{9}$$

where $k \in N$, T_{ext} is the period of the temperature increase stimulation, and T_d is the duration of the heat shock at 42 °C. $f_T(t)$ is equal to 1 during the heat shock and zero otherwise, which means that during the heat shock the σ^{32} concentration will increase.

Numerical values of the parameters used in the simulations shown in this paper are summarized in Table I. In order to take into account the stochastic nature of the cell processes, we include a multiplicative noise term in the half-life time γ_{pi} as well as in the proteins per mRNA ratio a_i of all genes,

$$\gamma_{pi} = \gamma_{pi}[1 + \xi(t)], \tag{10}$$

$$a_i = a_i[1 + \eta(t)], \tag{11}$$

where $\xi(t)$ and $\eta(t)$ are two independent Orstein–Uhlembeck noise terms (different for all cells), Gaussian distributed, with zero mean and correlation $<\xi(t)\xi(t')> = (A/\tau_c)e^{|t-t'|/\tau_c}$ [with $\eta(t)$ having equal correlation]. These noise terms are characterized by two parameters, the intensity A and the correlation time τ_c. The variance of the noise is given by A/τ_c, and hence we will measure its amplitude as $D = \sqrt{A/\tau_c}$. Correlation time is set to $\tau_c = 10$ min. In this way, noise strength can be adjusted through the parameter D, which in the simulations shown here ranges from 0.1 to 0.5. Equations are integrated with the Heun algorithm.[12] Similar results (not shown here) are obtained when multiplicative noise terms are introduced in γ_{mi} and α_i, and also with white noise terms instead of correlated noise.

TABLE I. Mean values of the parameters used in the numerical simulations.

	mRNA/transcript
α_i	0.5 mRNA/trans.
	Promoter leak
α_0	5×10^{-4} mRNA/trans.
	Number of protein for half repression of the promoter
K_0	40
	Proteins per mRNA per second
$a_i = a_{32}$	0.167 protein/mRNA/s
	Protein degradation constant
γ_{pi}	$\log(2)/(10\times60)$ min^{-1}
	σ^{32} degradation constant
γ_{32}	$\log(2)/(4\times60)$ min^{-1}
	mRNA degradation constant
γ_{mi}	$\log(2)/(60\times2)$ min^{-1}
	σ^{32} per transcript per second
k_{32}	0.02 trans./s

Finally, we introduce a parameter mismatch between cells, i.e., we consider a deviation of σ in the mean value of the parameter set (γ_{pi}, γ_{mi}, α_i, and a_i) within a population of N repressilators. These parameters will fluctuate at each cell in 100σ % around a mean value. For example, if $\sigma=0.1$ the numerical values of the parameter will be contained in a range of 10% around the mean value with a uniform probability. The same configuration of the genetic network has been modeled by a stochastic Gillespie[13] algorithm, obtaining qualitatively similar results.

Figure 2 shows the dynamics of a colony of $N=100$ repressilators in the absence of external perturbations. In the middle plot the protein number of CI, LacI, and TetR of one repressilator is displayed. Despite that we can see the oscillations of the three protein levels, they are not observable when measuring the mean value of one protein (e.g., LacI) for the whole population of repressilators if a certain parameter mismatch is considered, $\sigma=0.1$ in this case (see Fig. 2, bottom plot). Similar results were reported in Ref. 4, which leads to a serious drawback: the protein number of the whole colony is not oscillating.

III. ENTRAINMENT OF THE SYSTEM

At this point, we introduce an external periodic perturbation in order to synchronize the whole colony and, furthermore, to induce a desired frequency of oscillation in the global protein number.

Two aspects of the synchronization are studied here. First, the overall synchronization of the population is mea-

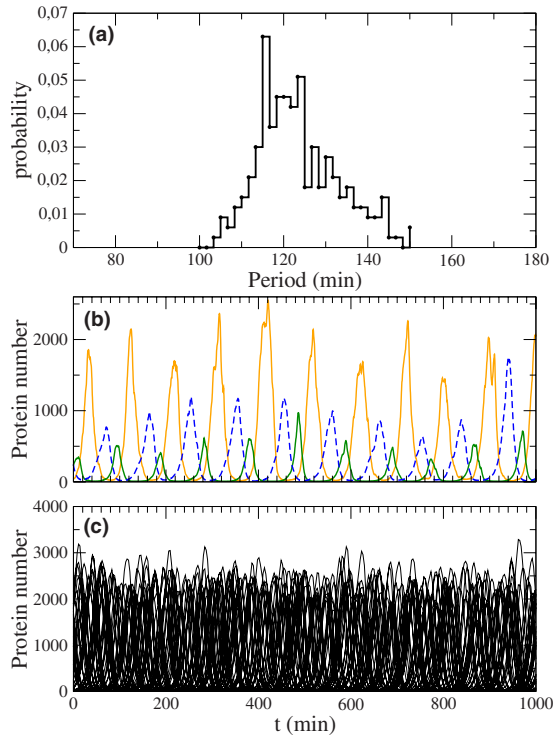

FIG. 2. (Color online) Dynamics of the cells in the absence of temperature variations. (a) Probability distribution of the periods of oscillation of the LacI protein for a population of $N=100$ cells. (b) Evolution of the CI (blue dashed line), TetR (green dark solid line), and LacI (orange light solid line) for a stable temperature of 36 °C. Noise level is set to $D=0.1$ and parameter mismatch is $\sigma=0.3$. (c) LacI protein number for the whole colony of bacteria.

sured quantitatively. With this aim, we compute the order parameter R, which is based on the variance of the mean field of a set of time series,[14]

$$R = \frac{\langle \overline{p_{2,j}}^2 \rangle - \langle \overline{p_{2,j}} \rangle^2}{\langle p_{2,j}^2 \rangle - \langle p_{2,j} \rangle^2}. \tag{12}$$

The brackets $\langle \cdots \rangle$ mean average over time while the horizontal bar $\overline{\cdots}$ means the average over the indices j such that

$$\overline{p_{2,j}}(t_0) = \frac{1}{N} \sum_{j=1}^{N} p_{2,j}(t_0). \tag{13}$$

In this equation $p_{2,j}$ refers to the time series of the second protein of the oscillator j. The parameter R is normalized in such a way that $R=0$ indicates a complete decorrelation between the time series, while $R=1$ reflects the complete synchronization between them.

Second, the mean frequency of the oscillations is compared to the frequency of the external perturbation. Following the work of Rosenblum *et al.*,[15] we evaluate the phase difference between two oscillators as they evolve in time. The phase of the oscillator is extracted with the Hilbert transform[16] and it is compared to the phase of the external signal in order to determine if the phase locking of the genetic oscillators occurs. The phase of the oscillators grows with time as $\Phi_j=\omega_j t$, while the phase of the external perturbation is $\Phi_{\text{ext}}=\omega_f t$. When phases of the repressilators are locked, the phase difference remains bounded in time,

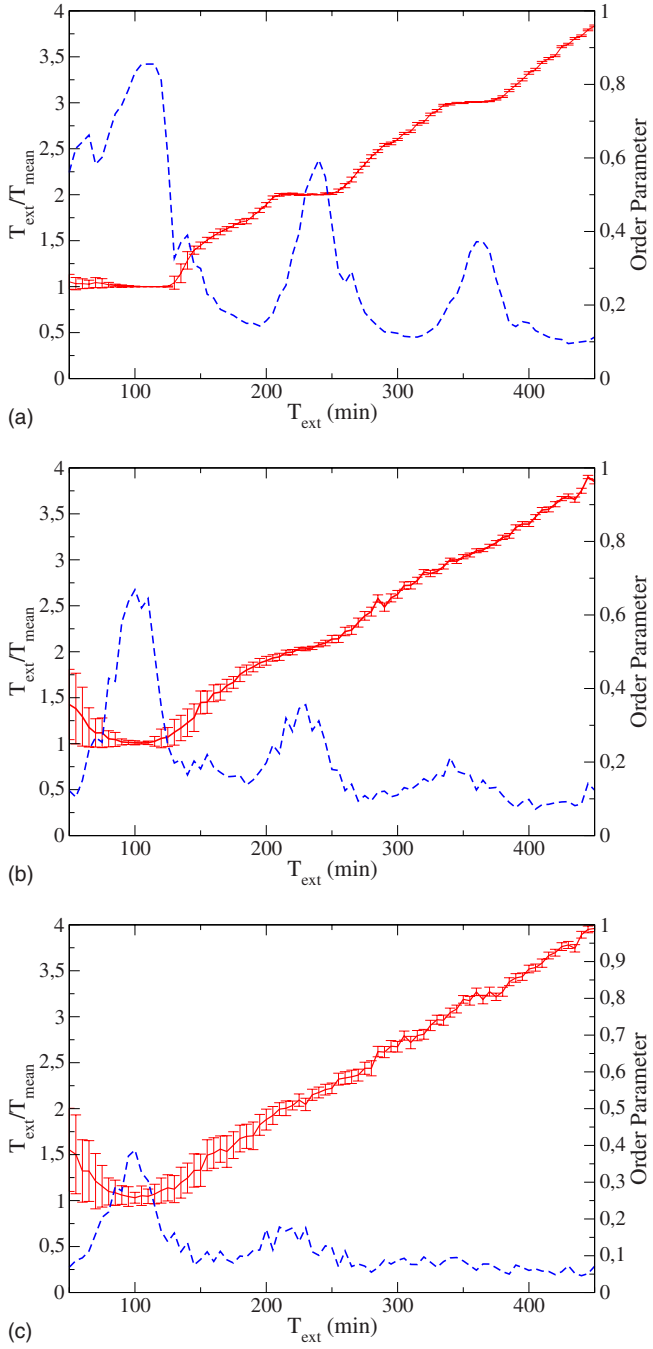

FIG. 3. (Color online) Entrainment of the system as a function of the external period. We plot the order parameter computed for the whole colony (dashed line, right scale) together with the ratio between the mean period of the system (T_{mean}) and the external period (T_{ext}) (solid line, left scale). Three different levels of the parameter mismatch are shown: (a) $\sigma=0.1$, (b) $\sigma=0.3$, and (c) $\sigma=0.5$. Noise level is set to $D=0.1$ in all cases.

$$|\Phi_{\text{ext}} - \Phi_j| < C, \tag{14}$$

with C being a constant, which indicates that $\omega_f=\omega_j$. On the other hand, when repressilators are evolving with different frequencies, the phase difference grows with time,

$$|\Phi_{\text{ext}} - \Phi_j| = \Delta\Omega t. \tag{15}$$

This technique allows to measure the ratio between the mean period of the oscillators T_{mean} and the forcing period T_{ext}.

Figure 3 shows the results of evaluating the quality of

synchronization for three different levels of noise for a colony of $N=100$ repressilators. A simulated heat shock is applied to all cells at every period T_{ext} with a duration of 5 min. Simulations are repeated for three different values of the parameter mismatch: $\sigma=0.1$ (upper plot), $\sigma=0.3$ (middle plot), and $\sigma=0.5$ (bottom plot). Blue lines correspond to the R parameter as a function of the period of the external perturbation (i.e., the period at which heat shock is applied). Three maxima appear, in all cases, at $T_{nat}\sim 120$ min, $2T_{nat}$, and $3T_{nat}$. They are harmonics of the mean period of the repressilators in the absence of external perturbation, what we call T_{nat}. When the system is perturbed at its natural (mean) frequency, we obtain the highest value of R for all mismatches and, furthermore, the ratio between T_{ext}/T_{mean} is close to unity, which indicates that the system is not only synchronized but follows exactly the period of the external forcing. In other words, the colony is *entrained* by the external frequency. The second highest peak corresponds to $T_{ext}\sim 2T_{nat}$, in this case, the order parameter is lower than the highest peak, which reveals that despite entrainment is achieved, it does not have the same quality as for T_{ext} close to T_{nat}. The ratio between the external and mean period is $T_{ext}/T_{mean}\sim 2$, indicating that the colony is oscillating at half of the external period, i.e., its natural period T_{nat}. The same phenomenon is reported at an external period of $T_{ext}\sim 3T_{nat}$, but in this case, with a drastic decrease in the quality of the entrainment. In fact, it is reasonable to expect a worsening of the order parameter when the period of the external forcing is increased, since the longest the external period, the longest the system is oscillating without a heat-shock induction.

IV. SYNCHRONIZATION VERSUS DESYNCHRONIZATION

Now we focus on the transition from the unsynchronized state to the synchronized state and vice versa. As the periodic stimulus is applied, the colony of cells may take some time to reach a stationary regime, that is, a state where the overall synchronization is maintained. On the other hand, if the periodic stimulus is switched off, the system may go through a transition from the synchronized state to a complete disorganized colony.

In order to evaluate the behavior of the system during transient times we compute the short time order parameter (STOP). The STOP gives a measure of the synchronization of a set of oscillatory systems over a time window T_0. This parameter is computed over a sliding time window for all the length of the time series and is given by

$$\mathrm{STOP}(t)=\frac{\langle\overline{p_{2,j}}^2\rangle_{T_0}-\langle\overline{p_{2,j}}\rangle_{T_0}^2}{\langle p_{2,j}^2\rangle_{T_0}-\langle p_{2,j}\rangle_{T_0}^2}, \tag{16}$$

where $\langle\cdots\rangle_{T_0}$ indicates time average over the time window and $\overline{\cdots}$ denotes average over the population of repressilators.

Figure 4 shows an example of the STOP value together with the number of the LacI proteins for a colony of $N=30$ cells. In this case, we let the colony to evolve freely up to $t=600$ min. Since a parameter mismatch of $\sigma=0.1$ and a noise of $D=0.1$ are considered, global oscillations of the protein number are not observed. Nevertheless, when the heat-shock response of the system is activated by periodic increases in the temperature (up to 42 °C) at a period of $T_{ext}=130$ min, synchronization in the protein fluctuations arises. Furthermore, it is maintained during the forcing interval, which goes from $t_{on}=600$ min to $t_{off}=2000$ min, and arriving to this point we turn off the temperature jumps and let the colony to evolve freely again. It is interesting to observe that the time needed by the system to synchronize is much shorter than what it takes to fully recover the unsynchronized state. Finally, in Fig. 5 we show the same phenomenon from another perspective, the raster plot of a colony of $N=100$ where $t_{on}=1000$ min and $t_{off}=2000$ min.

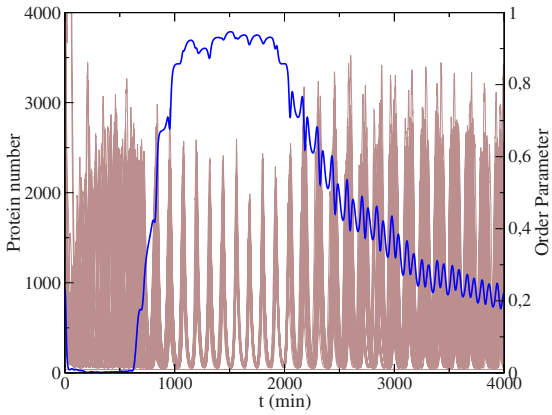

FIG. 4. (Color online) Evolution of the protein number for a set of $N=30$ cells and its corresponding STOP (dashed line, right scale). External modulation is applied only between the dashed lines, i.e., from $t_{on}=600$ min to $t_{off}=2000$ min. Other parameters of the simulation are $D=0.1$, $\sigma=0.1$, and $T_{ext}=130$ min.

In order to quantify the transition from the synchronized state to the unsynchronized state, we define the desynchronization time. We computed the STOP for several simulations with different values of the time window T_0, parameter mismatch, and noise level. We observed that in all cases, the

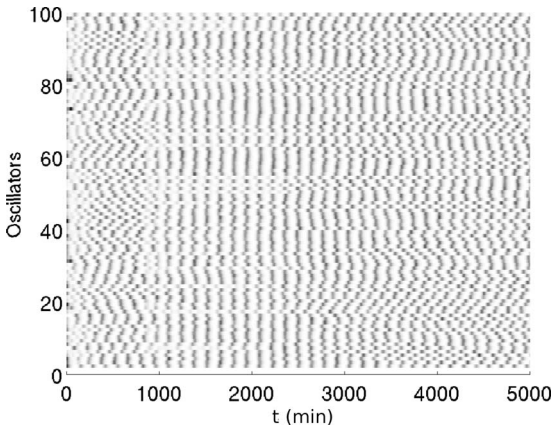

FIG. 5. Raster plot of a colony of $N=100$ cells. Each line represents the LacI number of one cell in a gray scale with black and white colors corresponding to local maxima and minima, respectively. External perturbation is applied at $t_{on}=600$ min and turned off at $t_{off}=2000$ min. The period of the temperature variations is set to $T_{ext}=130$ min. Other parameters are $D=0.1$, $\sigma=0.1$.

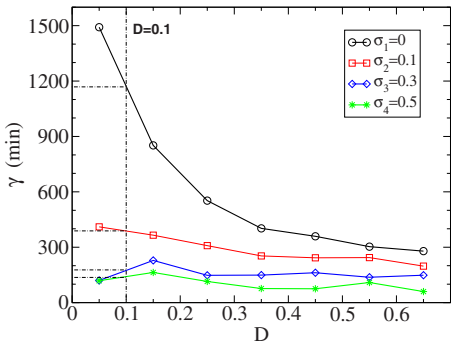

FIG. 6. (Color online) Desynchronization time γ as a function of the noise level D for four different parameter mismatches $\sigma_1=0.0$ (no mismatch), $\sigma_2=0.1$, $\sigma_3=0.3$, and $\sigma_4=0.5$. This figure illustrates the time that the system takes to evolve from a fully synchronized state to the unsynchronized state as a function of the noise and the parameter mismatch.

STOP decreases exponentially. Therefore, we fit the process of desynchronization of the colony with an exponential function given by

$$g(t) = r_0 + r_1 e^{-(t-t_{\text{off}})/\gamma} \tag{17}$$

for $t > t_{\text{off}}$ and $T_0 = 120$ min. We evaluate the parameter γ, which from now on we call the *desynchronization time*, with a nonlinear fitting method. We will focus on the influence of the noise level D on the desynchronization time. The fit is realized with an optimization function based on the simplex method. The parameters to evaluate are γ, r_0, and r_1. The optimization function searches the local minimum of the function

$$fobj(r_0, r_1, \gamma) = \sum_{k=t_0/T_e}^{t_1/T_e} (g(kT_e) - \text{STOP}(kT_e))^2, \tag{18}$$

where T_e is the sampling period of the signal. The evaluation of the desynchronization time as a function of the noise level, and for three different parameter mismatches, is summarized in Fig. 6. As expected, the transition from the synchronized to the unsynchronized state depends on both D and σ. For a given value of D (e.g., $D=0.1$, dashed line of Fig. 6) it is possible to estimate the desynchronization time. Once it has been estimated, the ranges of the external periods that can be applied to entrain the system are limited by the obtained desynchronization time. When $T_{\text{ext}} > \gamma$, the period of forcing is so slow that the system has enough time to unsynchronize before the next temperature stimulus, that is, the population does not maintain the synchrony during the time between two external pulses. On the contrary, it is reasonable to expect a good entrainment of the system when external modulation periods are lower than the desynchronization time. Dashed lines of Fig. 6 indicate the desynchronization time for the different parameter mismatches (and a fixed noise level $D=0.1$) corresponding to Fig. 3. Comparing both figures, we can observe that external periods higher than the desynchronization time lead to low values of the order parameter.

Since in our particular case oscillators are not coupled with each other, the synchronized motion of the protein levels is due to a frequency locking by means of a periodic external action.[17] When the frequency of the oscillators lies inside the synchronization region and noise amplitude is bounded (which is the case of our system), phase locking is achieved, despite small fluctuations induced by noise are observed around the mean phase. Nevertheless, when external forcing is interrupted, the phases of the oscillators begin to diverge due to the joint action of noise and parameter detuning. Since oscillators have independent motions in the absence of external driving, the phase diffusion is simply due to differences in the natural phase of the oscillators [see Fig. 2(a)] and the intrinsic noise of the system.

V. CONCLUSIONS

In the present report we propose a modification of the synthetic genetic network known as the repressilator in order to enhance the production of one of its proteins by a heat shock mechanism, which is activated in *E. coli* by exposure to high temperatures. Thus, we are able to entrain the oscillations of a colony of repressilators by periodically modulating the temperature. Our numerical observations show regions of entrainment, which are related to the natural frequency of the modified repressilator. In addition, we have seen that when the external stimulation is turned off, the system requires a much longer period of time in order to loose the synchronized state than the time required to be synchronized. This fact reveals the robustness of the entrainment method and suggests modulation techniques where the temperature shifts are not applied continuously. These results would motivate the verification of the model proposed here in experiments performed in real bacteria containing the proposed modification of the repressilator, in order to evaluate its usefulness in biotechnological large-scale production.[18] In fact, preliminary heat shock assays performed with bacteria transformed with the original nonmodified repressilator suggests synchronization of bacteria population in response to heat shock. Regarding this point, the introduction of the repressilator system induced by heat shock onto production strategies may lead to cost-reducing, time-saving, and efficiently regulated production without chemical induction of the culture.[19] Replacing GFP reporter gene from the pZE plasmid of the original repressilator by a recombinant gene coding for a product of interest and suitable for being exported to culture media could avoid lysis of synchronized and highly efficient productive culture. When synchronized culture is in nonproduction phase by repression of tetR promoter, culture media containing product of interest could be replaced by fresh media, avoiding need of cell lysis. The obtained medium could be processed for product purification; meanwhile synchronized culture is in productive phase inside the fermentation tank. In a more general framework, we have provided a particular example of the possibilities of synthetic biology; nevertheless it is necessary to develop new models and techniques that help us to understand the complexity of living systems.

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