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Synthetic multicellular oscillatory systems: controlling protein dynamics with genetic circuits

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Abstract
Synthetic biology is a relatively new research discipline that combines standard biology approaches with the constructive nature of engineering. Thus, recent efforts in the field of synthetic biology have given a perspective to consider cells as 'programmable matter'. Here, we address the possibility of using synthetic circuits to control protein dynamics. In particular, we show how intercellular communication and stochasticity can be used to manipulate the dynamical behavior of a population of coupled synthetic units and, in this manner, finely tune the expression of specific proteins of interest, e.g. in large bioreactors.

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(Some figures in this article are in colour only in the electronic version.)

1. Introduction: the potential of synthetic biology for controlled protein production

In the past decade, synthetic biology has revolutionized the concepts and approaches of engineering biological systems. The growing understanding of cellular processes and the potential that molecular biology techniques offer nowadays have provided a solid basis for the development of this research discipline. Its main focus is on the design of sophisticated synthetic genetic circuits which should, in the near future, be capable of controlling gene expression, information processing, communication, etc.

In general, synthetic biology approaches the problems from a novel, engineering perspective, which has already led to the development and construction of various synthetic devices and small circuit modules capable of performing predefined tasks. Currently, these circuits include switches (Gardner *et al* 2000, Kramer *et al* 2004, Ham *et al* 2008), cascades (Hooshangi *et al* 2005), pulse generators (Basu *et al* 2004), oscillators (Elowitz *et al* 2000, Atkinson *et al* 2003, Stricker *et al* 2008, Tigges *et al* 2009), logic gates (Rinaudo *et al* 2007), etc. These synthetic units are not derivatives of natural circuits, but are engineered designs that could function independently of the host cell and therefore offer the opportunity to study specific functions and signaling pathways for which limitations occur in the natural environment. Thus, these circuits can serve to control gene expression, protein function or metabolism. Additionally, most of the existing units can also reproduce or mimic given cellular behavior. This, on the other hand, offers the possibility of gaining valuable information about the design and functionality of natural genetic circuits.

However, most of the circuits designed so far have been fairly simple and directed generally at executing specific functions in isolated cells. This could further lead to controlled isolated behavior (e.g. the synthesis of a specific protein can be regulated if the expression of the corresponding gene is placed under the control of a synthetic circuit—a

switch or an oscillator). The practical application of the synthetic modules, on the other hand, requires a larger functional synthetic system, which could be programmed to execute various tasks in a cellular environment. Thus, the challenges that the synthetic biology target disciplines (such as chemical and pharmaceutical engineering) are imposing on current research in this field necessitate substantially new design principles and an improved scientific understanding of the biological environment. This implies the development of means of (synthetic/artificial) communication between separate cells, as a way to partially bridge the problem of regulated dynamical behavior of a cellular population. The design of multicellular systems that can exhibit a finely tuned coordinated behavior is a major actual challenge for synthetic biology (McMillen *et al* 2002, García-Ojalvo *et al* 2004, Ullner *et al* 2007, Danino *et al* 2010). By constructing and analyzing synthetic multicellular systems that use artificial signaling, we can address the question of controlling a population's dynamical behavior and, in this manner, address the question of controlling the expression of proteins of interest. We have shown recently (Koseska *et al* 2007a, Ullner *et al* 2008, Koseska *et al* 2010) that multi-stability and multi-rhythmicity are inherited properties of synthetic circuits coupled via a specific type of quorum-sensing mechanism, allowing the synthetic system to exhibit high adaptability, as is typical of natural systems.

Following this line of research, here we show how intercellular communication can be used to control protein dynamics. The subject of our study is a system consisting of synthetic oscillators (Kuznetsov *et al* 2004) coupled via an intercellular mechanism, which in turn is able to establish controlled expression of constant proteins with strictly regulated concentration values. The usage of oscillating units for the control of protein production is important, since a vast range of proteins that govern fundamental physiological processes, such as insulin secretion (Tsaneva-Atanasova *et al* 2006), cell cycle and circadian rhythms (Lloyd *et al* 1990, Gonze *et al* 2005, Locke *et al* 2005), display oscillatory behavior. Moreover, the control of protein production we propose here does not depend on the topological structure of the system: we investigate regulatory mechanisms in globally as well as in locally coupled synthetic systems. The latter allows also a spatial, in addition to the dynamical, regulation of the protein production. In the context of gene expression regulation, we also discuss the necessary conditions under which noise can regulate the dynamical behavior of the system.

2. Model of the synthetic multicellular system

We consider a model of hysteresis-based relaxation genetic oscillators coupled via a quorum-sensing mechanism (Kuznetsov *et al* 2004). The oscillator is constructed by combining two engineered gene networks: the toggle switch (Gardner *et al* 2000) and an intercell communication system (Kobayashi *et al* 2004, Fuqua and Greenberg 2002). The synthesis of the two repressor proteins, which constitute the toggle switch, is regulated in such a way that the expression of both genes is mutually exclusive, and organizing bistability. The second network is based on the dynamics of an autoinducer (AI), which, on the one hand, drives the toggle switch through a hysteresis loop and, on the other hand, provides intercell communication by diffusion through the cell membrane.

The time evolution of the elements in the system is governed by the following dimensionless equations (for details, see Kuznetsov *et al* (2004)):

$$\frac{\mathrm{d}u_i}{\mathrm{d}t} = \alpha_1 f(v_i) - u_i + \alpha_3 h(w_i), \tag{1}$$

$$\frac{\mathrm{d}v_i}{\mathrm{d}t} = \alpha_2 g(u_i) - v_i, \tag{2}$$

$$\frac{\mathrm{d}w_i}{\mathrm{d}t} = \varepsilon(\alpha_4 g(u_i) - w_i) + 2d(w_\mathrm{e} - w_i), \tag{3}$$

$$\frac{\mathrm{d}w_\mathrm{e}}{\mathrm{d}t} = \frac{d_\mathrm{e}}{N}\sum_{i=1}^{N}(w_i - w_\mathrm{e}). \tag{4}$$

where N is the total number of cells (oscillators), u_i and v_i represent the proteins from which the toggle switch is constructed in the ith cell and w_i represents the intracellular and w_e the extracellular AI concentration (figure 1). The mutual influence of the genes is defined with the following functions:

$$f(v) = \frac{1}{1+v^\beta}, \quad g(u) = \frac{1}{1+u^\gamma}, \quad h(w) = \frac{w^\eta}{1+w^\eta},$$

where β, η and γ are the parameters of the corresponding activatory or inhibitory Hill functions.

In equations (1)–(4), the dimensionless parameters α_1 and α_2 regulate the repressor operation in the toggle switch, α_3 the activation due to the AI and α_4 the repressing of the AI. The coupling coefficients in the system are given by d and d_e (intracellular and extracellular) and depend mainly on the diffusion properties of the membrane, as well as on the ratio between the volume of the cells and the extracellular volume (Kuznetsov *et al* 2004). If the parameter ε is small ($\varepsilon \ll 1$) (Kuznetsov *et al* 2004), as in our case, the evolution of the system splits into two well-separated timescales: a fast dynamics of u_i, v_i and a slow one of w_i. Due to this presence of multiple timescales, the system can produce relaxation oscillations.

The intercell signaling in this model is organized through the slow recovery variable. As is known from oscillation theory, such coupling has phase-repulsive properties and can be referred to as inhibitory. However, such organization is not only characteristic of globally coupled systems, but can also be realized in a system where the coupling has local properties. We envision, for example, a set of synthetic oscillators organized in a ring, where the following equation substitutes equations (3) and (4) from the previous model:

$$\frac{\mathrm{d}w_i}{\mathrm{d}t} = \varepsilon(\alpha_4 g(u_i) - w_i) + 2d(w_{i+1} + w_{i-1} - 2w_i). \tag{5}$$

Such spatial organization of the synthetic units has a significant influence on the dynamics of the system, which we investigate next.

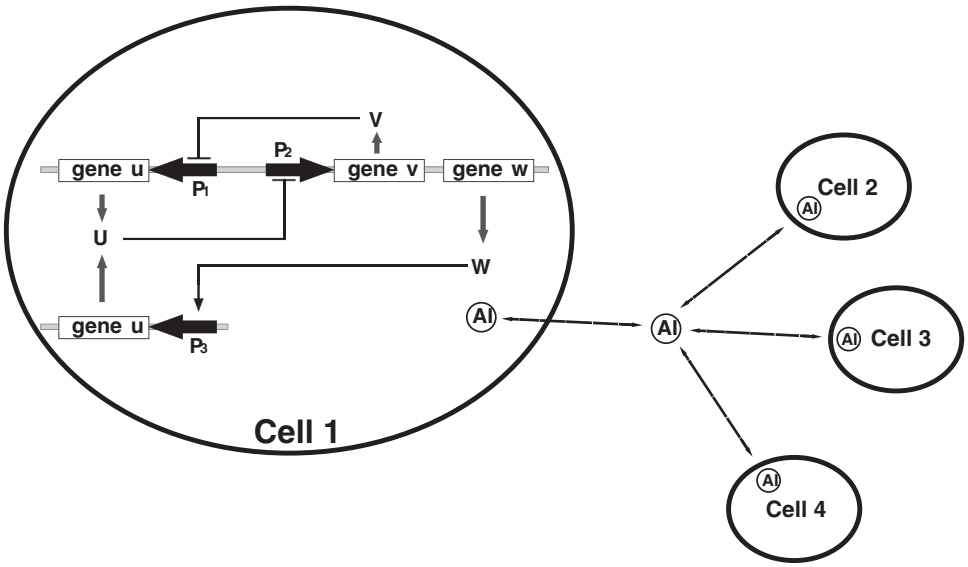

Figure 1. Schematic diagram of the network of genetic relaxation oscillators. u, v and w denote the genes and P_1, P_2 and P_3 the corresponding promoters. AI refers to the AI molecules.

3. Results and discussion

3.1. Control of protein production in a population of identical synthetic circuits

In our previous work (Koseska *et al* 2007a), we have shown that multi-rhythmicity and coexistence of several attractors is a typical property of the globally coupled system (equations (1)–(4)). The main manifestation of multi-stability for systems of globally coupled oscillators is clustering, defined as a dynamical state of the system characterized by the coexistence of several subgroups, where the oscillators exhibit identical behavior (Golomb *et al* 1992, Okuda *et al* 1993). Here, two separate cluster formations are possible: steady state and oscillatory clusters (for a detailed explanation, see Koseska *et al* (2007a)). In this work, we will mainly elaborate on the steady state clusters, and in particular the oscillation death (OD) phenomenon, since it is responsible for the production of constant levels of protein concentration in an oscillating population.

The OD phenomenon was initially found by Prigogine and Lefever (1968) for two identical Brusselators coupled in a diffusion-like manner. Their interaction can break symmetry (via a pitchfork bifurcation), which leads to a stable inhomogeneous steady state (IHSS). Furthermore, it has been shown theoretically that OD is model independent, persisting for large parametric regions in several models of diffusively coupled chemical (Bar-Eli 1985) or biological oscillators (Tsaneva-Atanasova *et al* 2006, Kuznetsov *et al* 2004, Koseska *et al* 2007a, Ullner *et al* 2007, Shpiro *et al* 2007). Experimental results reported by Dolnik and Marek demonstrate the extinction of oscillations in chemical reactors coupled by mutual mass exchange (Dolnik and Marek 1988). Later, Crowley and Epstein demonstrated for two coupled, slightly nonidentical chemical oscillators that the basis for the OD is a specific, vector-type coupling, namely coupling via a slow recovery variable (Crowley and Epstien 1989). Very recently, OD has been experimentally observed in chemical nano-oscillators (microfluidic Belousov–Zhabotinsky octane droplets), diffusively coupled via signaling species (Br2 in this case) (Toiya *et al* 2008). Moreover, the formation of OD phenomena in discrete arrays of coupled nonlinear cells has been also discussed in relation to stable nonuniform spatial pattern formation, when independent cells have only a unique stable state. In this context, it was shown that OD can arise in a circular array whose group of symmetries is the dihedral group D_{2n} of the regular $2n$-sided polygon. Thus, the symmetric patterns (equivalent to the two branches of the OD solution) can be determined by a linear analysis of the D_{2n} symmetry of a circular array (Epstein *et al* 1993).

Identical oscillators engaged in OD in the synthetic system we analyze here are distributed between two clusters, each of them being in a steady state, which corresponds to two different but constant protein levels. For a synthetic system of N coupled cells, we have previously found the existence of $(N-1)$ possible different distributions of the oscillators between these two clusters, each characterized by a shift in the protein production level (Koseska *et al* 2007a), as shown in figures 2(a) and (b).

Thus, the production of specific protein concentration levels can be controlled via the dynamical behavior of the population and the possibility for clustering, when using diffusive coupling realized through the slow variable as a mechanism for communication between distinct synthetic units. Moreover, the percentage of cells distributed in the lower and/or in the upper OD cluster depends on the parameter choice: lower α_1 values for which OD is established ensure the majority of cells to produce lower concentration values and, vice versa, high α_1 values allow most of the oscillators to populate the upper clustering level, and in this way, 'direct' the production of, in particular, high concentration values. The possibility of fine control over the protein production in interacting cellular populations using only one of the parameters (here we use α_1) in the system opens up a novel horizon in synthetic biology research: one could engineer a synthetic system where not only the concentration levels could be manipulated, but also

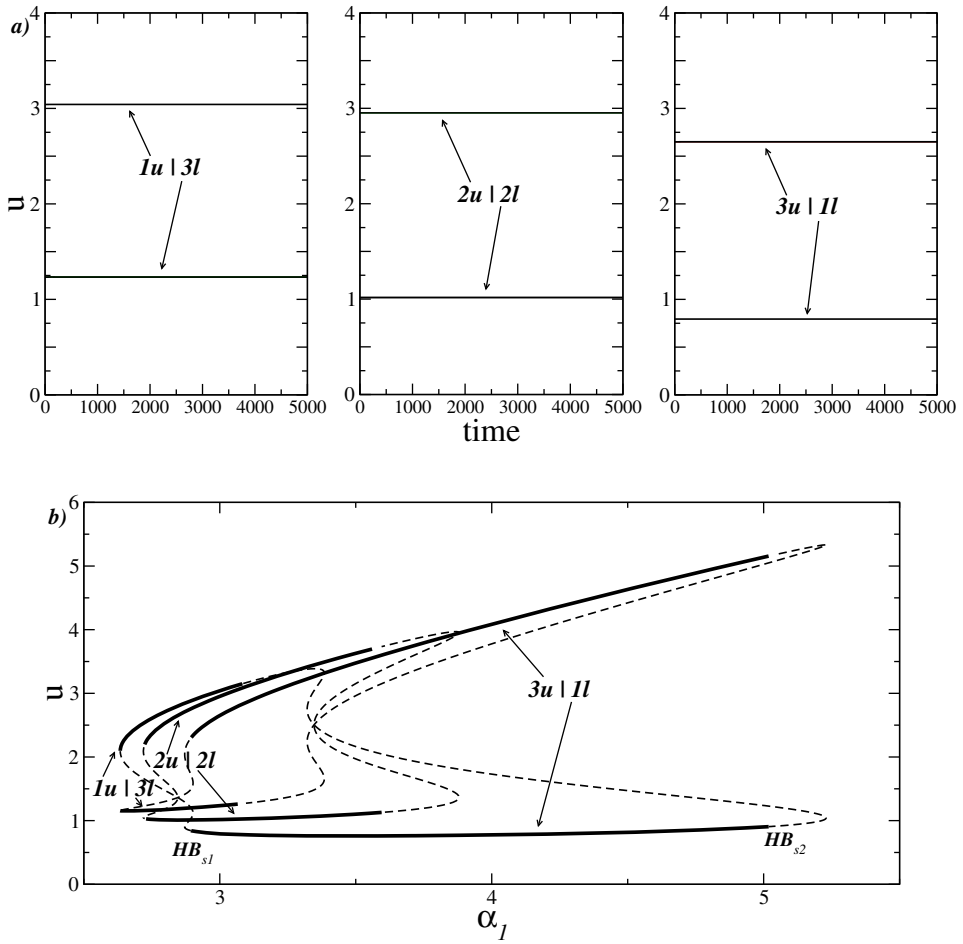

Figure 2. (a) Examples of different distributions of the oscillators in the steady state clusters for a system of $N = 4$ cells (for the system of equations (1)–(4)). From left to right: distribution $1u|3l$, where 1 oscillator occupies the upper level and 3 the lower one; distribution $2u|2l$; and distribution $3u|1l$. Note the different protein levels for different oscillator distributions. Parameters: $\varepsilon = 0.01$, $\alpha_1 = 3$, $\alpha_2 = 5$, $\alpha_3 = 1$, $\alpha_4 = 4$, $\beta = \eta = \gamma = 2$, $d = 0.3$ and $d_e = 1$. (b) Bifurcation chart showing the stable cluster decompositions in the OD regime for the system of $N = 4$ elements. Each of the OD clusters is stabilized via a Hopf bifurcation, HB (for simplicity, here we denote the positions of the HBs for only one of the clusters). Note that the bifurcation chart is not complete, depicting only branches where stable OD cluster decompositions exist. Here and in the following charts, thin solid lines denote the homogeneous steady state (HSS), thick solid lines denote the stable inhomogeneous steady state (OD) and dashed lines denote the unstable steady state.

the percentage of cells in the population producing specific protein concentrations.

3.2. Control of protein dynamics in a heterogeneous population

Through the investigation of coupled identical units, we have shown that synthetic circuits in the OD mode are a promising tool for cell function regulation, because they can provide for stable diversity in protein production. However, identical cells represent a limiting case of studying biological systems, since almost all biological processes, especially those that occur on a genetic level, are noisy in general. Thus, many regulatory parameters differ when comparing separate cells. Therefore, we show the necessary conditions for OD occurrence and, in this manner, the regulation of protein production in a synthetic system of non-identical elements, as an example of natural heterogeneity. Since the dynamic behavior of the circuit is regulated by α_1, we assume that the detuning between different cells is expressed in the variability of the α_1 parameter values, thus defining the detuning measure between different cells as $d_{ij} = \alpha_1^{(i)}/\alpha_1^{(j)}$. α_1 determines the expression strength of the gene and is proportional to the concentration of plasmids present in the cell; subsequently, it can be manipulated experimentally (Paulsson *et al* 2001). For the generalized case of N coupled non-identical synthetic units, as shown in Koseska *et al* (2009), the possibility of controlling the production of specific constant protein levels is still present, since the effect of clustering is ensured via the phase-repulsive coupling. For OD, the system demonstrates again two cluster decompositions, independently of N. Moreover, OD dominates over given parameter ranges (in the form of cluster formation), 'pushing' the oscillatory solutions between the stable OD branches (figure 3). This phenomenon, called oscillation death dominance (ODD), as reported in Koseska *et al* (2009), can be additionally seen as a powerful regulator of the synthetic networks' dynamics in the case of strong coupling.

It is important to note here that the OD phenomenon that we discuss in this work is significantly different from other types of coupling-dependent quenching of oscillations, which are classified under amplitude death (AD) phenomena (Zhai *et al* 2004, Herrero *et al* 2000) (the same is valid for ODD and partial AD phenomena (Atay 2003b)). It has been proven

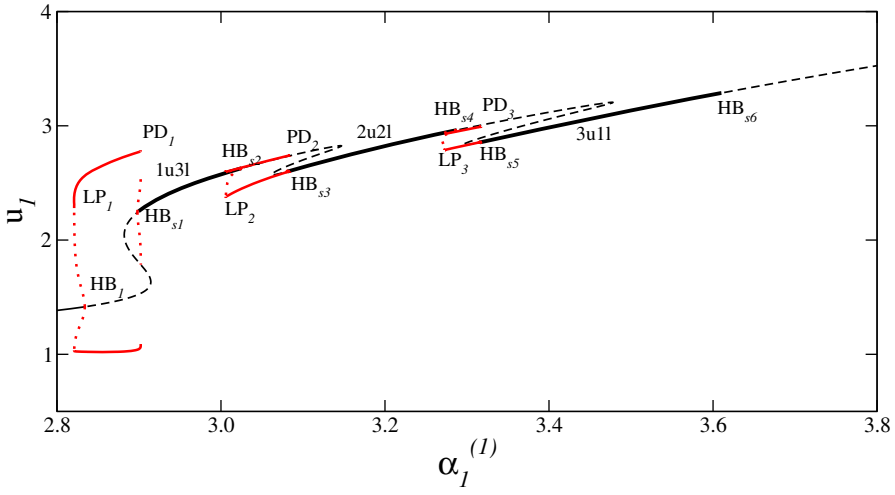

Figure 3. Different stable cluster distributions for $N = 4$ coupled non-identical oscillators (the bifurcation branch for one oscillator is plotted). From left to right: 1 oscillator located in the '(**u**)pper' OD cluster and 3 in the '(**l**)ower' one—1*u*3*l* distribution; 2*u*2*l*; and 3*u*1*l* distribution. The oscillatory solutions (asymmetric oscillations) are 'pushed' between the stable distributions, establishing OD dominance. Parameters: $\alpha_1^{(2)} = 2.592$, $\alpha_1^{(3)} = 2.646$, $\alpha_1^{(4)} = 2.565$ and $d = 0.007$. The solid (red) lines denote the stable limit cycle (stabilized between a limit point (LP) and a period doubling bifurcation (PD)) and the dotted lines denote the unstable limit cycle.

that for sufficiently strong coupling and sufficiently large variance of the distribution of the frequencies, the oscillators pull each other off their limit cycles and into the origin, a stable equilibrium point (Mirollo *et al* 1990), which is called AD. Moreover, it has been shown that AD, in contrast to OD, is stable also for delayed coupling (scalar or vector) (Ramana Reddy *et al* 1998, Atay 2003a). Thus, AD results in a *homogeneous* steady state (all oscillators in the system display identical steady state behavior), despite the OD phenomenon, which, as mentioned, is characterized by distinct steady state levels. OD is, on the other hand, important from a biological perspective, since it provides a stable heterogeneity in a homogeneous medium. Here, we point out once again that both the cluster distributions through which OD is manifested (the upper and the lower steady state) are always stable in the same parameter region. In contrast to this, in the general bistable systems, the system can switch from the first to the second stable state, which need not be symmetric with respect to the stored energy.

3.3. Regulation of protein dynamics in systems with distinct topology

The study of collective dynamical behavior of coupled nonlinear oscillators has proven to be successful in describing and understanding the properties of complex systems in general. Moreover, in order to investigate and analyze the functionality of complex networks, it is important to understand the dynamics of basic building blocks with a specific topology, since this enables the control not only in the phase space, but also in the real space. It is known that the diffusive coupling mechanism organized locally ensures the presence of various rhythmogenic activities in the system (Rabinovich *et al* 1999, Yang *et al* 2002, Koseska and Kurths 2010). Thus, we investigate such mechanisms through which control over gene expression can be established in a ring of locally coupled synthetic oscillators (detailed model described via equations (1), (2) and (5)).

In this case, the number of stable dynamical regimes increases significantly in contrast to the globally coupled case. As a reminder, we have demonstrated in Koseska *et al* (2007a) that the number of stable regimes for globally coupled systems is dependent on the coupling strength d: for $d < 0.006$, three stable regimes are present (in-phase, anti-phase and asymmetric), whereas for larger coupling values ($d > 0.006$), OD and in-phase oscillations are the only stable solutions. Increasing the size of the system (the number of oscillators) does not influence the number of stable regimes, but is rather responsible for the presence of additional stable attractors that appear as a result of the possibility for different distributions of the oscillators between different clusters in the anti-phase, asymmetric and OD regimes (as shown in figure 2, for example). Considering signaling mechanisms with local characteristics, on the other hand, leads to the presence of an increased number of stable regimes. Despite the coupling strength dependence, this number is strongly influenced by the number of oscillators present on the ring, which makes the complete classification of existent dynamical regimes almost impossible to perform. Therefore, by means of detailed bifurcation analysis, we attempt to characterize a limited example here, namely a system of $N = 8$ identical oscillators, locally coupled on a ring. For small coupling values (e.g. $d = 0.002$), we identified four separate regimes as stable, an OD regime and three distinct oscillatory solutions (the complete bifurcation diagram and the time series of OD and two of the oscillatory solutions are given in figure 4).

The OD regime (stabilized between two Hopf bifurcations, $HB_{s1/2}$, as shown in figure 4(a)) is in this case manifested as well in the standard, two-cluster decomposition (figure 4(b)), and is a result of symmetry breaking of a system via a pitchfork bifurcation. A different distribution of the oscillators between the two clusters is again possible, resulting in $N - 1$ separate stable attractors (as for globally coupled systems). In contrast to the globally coupled case however (where the presence of two Hopf bifurcations was

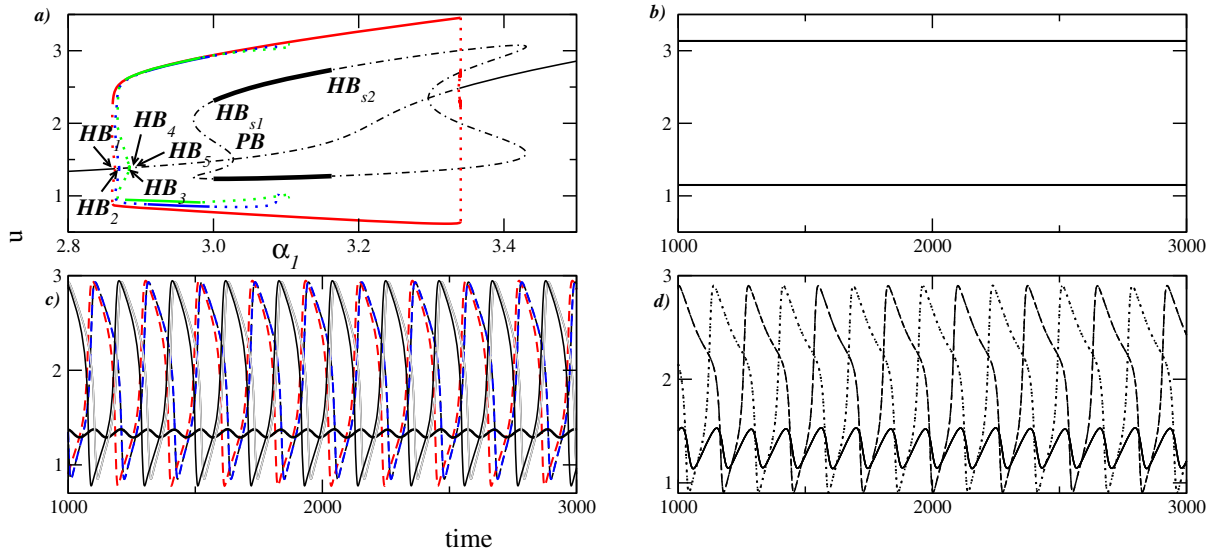

Figure 4. Bifurcation structure for a ring of $N = 8$ oscillators, locally coupled (different colors denote different clusters). $d = 0.0002$ and (b) $\alpha_1 = 3.131$, (c) $\alpha_1 = 2.982$ and (d) $\alpha_1 = 3.001$. Other parameters are as in figure 2.

identified), here we have detected five Hopf bifurcations, three of them giving birth to stable limit cycles. In particular, HB_1 gives birth to a stable in-phase oscillatory regime, whereas the limit cycles arising from HB_2 and HB_3 are characterized by complex, asymmetric types of solutions. In figure 4(c), a five-cluster decomposition oscillatory solution arising from HB_2 is shown, with a distribution $2:1:2:1:2$ between different clusters, and figure 4(d) shows the oscillatory solution arising from HB_3. This regime is also characterized by the presence of large- and small-amplitude oscillations in one attractor. From the three-cluster decomposition that is observed, two of the clusters perform large excursions and oscillate in anti-phase, while the other one oscillates near the steady state with small amplitude. The distribution of the oscillators between the separate clusters is $3:3:2$. Another characteristic feature of this regime is the presence of synchronization of the order of $2:1$ between the asymmetric clusters. Again, different distributions of the oscillators between different clusters in both asymmetric oscillatory regimes are possible, resulting in different periods and amplitudes of oscillation of the distinct clusters (results not shown here).

Increasing the coupling strength in the case of locally coupled oscillators leads to the appearance of novel manifestation of the OD regime. In particular, for $d = 0.006$, despite the standard, two-cluster decomposition, we observe OD manifested as three- and five-cluster decompositions (figures 5(b) and (c)). As seen from the bifurcation chart, both solutions are the result of a symmetry breaking of the system via two separate pitchfork bifurcations (PB_1 and PB_2, in figure 5(a)). The three-cluster decomposition (figure 5(b)), stable between $HB_{s1/2}$, has a distribution $4:2:2$ (of the oscillators between distinct clusters). HB_{s3} and HB_{s4}, on the other hand, mark the stability of the five-cluster manifestation of OD. Here, the 'upper' level consists of three and the 'lower' of two separate clusters, with an end distribution of $1:2:2:2:1$ oscillators between different levels. These are only two examples of the possible manifestation of the inhomogeneous steady state in the ring structure, which are stable with respect to small perturbations. The number of possible OD manifestations increases with increasing coupling and the number of oscillators present in the system. This, in turn, contributes to an increased variability in the synthetic network. However, due to the specific topological structure, separate cells are localized in space. Thus, a clear control of the protein production in the system can be established.

3.4. Stochastic variability in systems of globally coupled synthetic units

Experimental evidence shows that among the most important factors affecting the performance of a cellular system within a living organism are intercellular communication (McMillen *et al* 2002) and noise (Elowitz *et al* 2002, Swain *et al* 2002). Moreover, the inherent stochasticity of biochemical processes, which depend on relatively infrequent molecular events involving a small number of molecules, is an essential source of internal noise in biological systems. Additionally, fluctuations originating from a random variation of one or more externally set control parameters act as external noise. Since the presence of noise is inevitable, the study of its impact on the dynamics of the gene network is, of course, very important. Thus, we explore here not only the effects that noise has on the protein dynamics, but also the question as to whether noise can contribute as a regulating factor in a synthetic system, i.e. the constructive effects of noise.

For this purpose, the noise influence on the systems' dynamics is represented via the noise term ξ, which models the contribution of random fluctuations and is a Gaussian white noise with zero mean. We consider noise intensity that is rather small, not exceeding the order of 10^{-2}, hence a sufficient motivation to use Gaussian noise and Langevin equations. Thus, the contribution of the noise to the dynamics of the system is modeled via

$$\frac{dw_i}{dt} = \varepsilon(\alpha_4\, g(u_i) - w_i) + 2d(w_e - w_i) + \xi_i(t). \qquad (6)$$

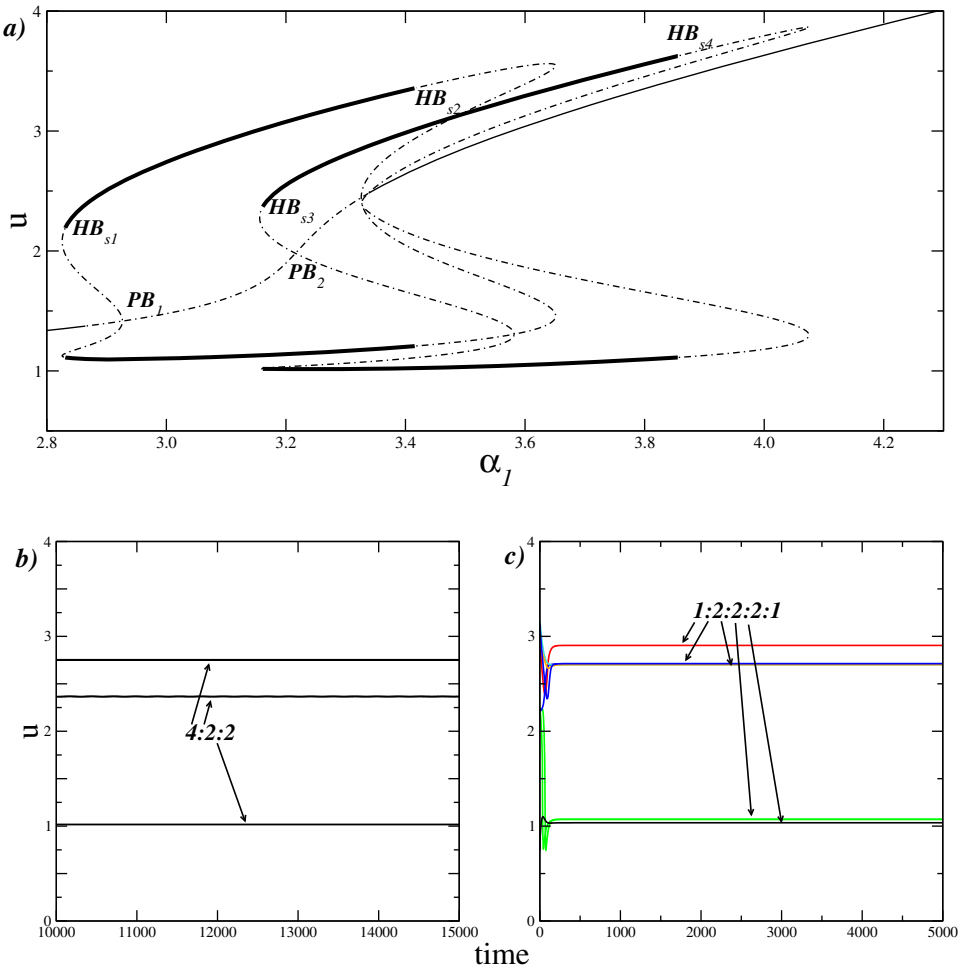

Figure 5. (a) Bifurcation chart displaying two different stable OD distributions. Manifestation of OD as a (b) three-cluster ($\alpha_1 = 3.161$) and (c) five-cluster ($\alpha_1 = 3.1$) decomposition (different colors denote different clusters). $d = 0.0006$ and other parameters as in figure 2.

Numerical integrations are performed using standard techniques for stochastic differential equations (García-Ojalvo *et al* 1999).

In systems where multiple stable attractors exist, noise can induce a robust switching between them. For example, a short noise pulse (with a duration of approximately 100 time units) can induce a stable switching between different cluster distributions in the OD regime. Due to the presence of $N-1$ possible stable distributions between the two clusters through which OD is, in general, manifested, a noise pulse with intensity of the order of 10^{-3} will enable a robust transition between them, e.g. from $2u|2l$ to $3u|1l$ distribution in the case of $N = 4$ coupled oscillators, as shown in figure 6(a). This provides an effective control mechanism to manipulate the percentage of cells that produce proteins with a given concentration. Moreover, manipulation of the noise pulse intensity can further lead to a transition between different dynamical regimes: due to the coexistence of OD and in-phase regime in a population of globally coupled identical oscillators, a noise pulse of the order of 10^{-2} could switch the production from constant (OD) to oscillatory protein concentrations (figure 6(b)).

As we have seen here (and shown previously in Koseska *et al* (2007b)), the presence of multi-stability strongly influences the response of the synthetic system to different external stimuli, such as the effects of extrinsic and intrinsic noise. Thus, complex dynamical behavior can be predicted as a result of the interplay between noise, heterogeneity and intercell coupling. We observe now the continuous influence of stochasticity on the behavior of the globally coupled synthetic units, when the system finds itself in the vicinity of the Hopf bifurcation (HB) through which the OD is stabilized (figure 2(b); HB_{s1}). In order to determine the effective jumps of the oscillators in the system due to noise, we analyze statistically the interspike intervals (ISIs), also called the frequency distribution (Klevecz 1976).

When the system is close to the HB through which the OD is stabilized, a constant noise influence on the system significantly contributes to enhancement of variability and the well-expressed presence of multiple frequencies: the solutions are now polymodal, as shown in figure 7, where the ISI distributions are shown for two separate oscillators from a system of $N = 8$ slightly inhomogeneous coupled synthetic units. Choosing slightly different α_1 values (the difference between the α_1 values in distinct cells is not larger than 4%), one can effectively switch between different multi-peak distributions, adapting the artificial network to produce the desired frequencies.

In general, we can state that in a broad parameter interval, control is saved in the presence of noise. Thus, we can speculate that noise selects the most robust distribution of cells in the clusters. However, due to variability in

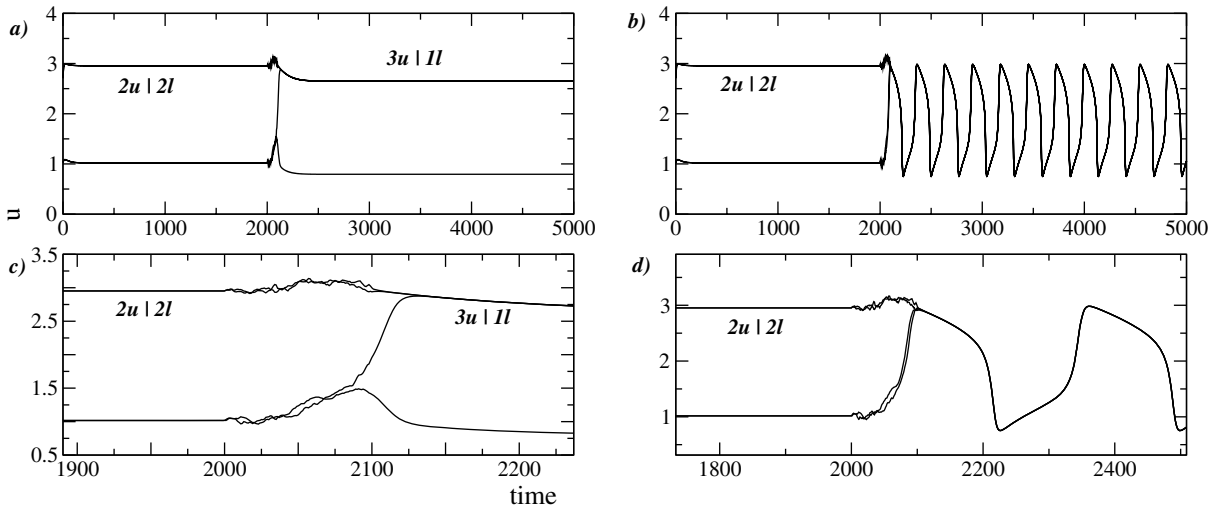

Figure 6. Robust switching between stable attractors due to a short noise pulse. (a) Transition between stable OD cluster distributions (from $2u|2l$ to $3u|1l$), for $N = 4$ and $\sigma_a^2 = 5.5 \times 10^{-3}$, and (b) switching between different dynamical attractors—OD and in-phase regime, for $N = 4$ and $\sigma_a^2 = 6 \times 10^{-2}$. Other parameters are as in figure 2. Detailed view of the transition (switching) between the (c) stable OD cluster distributions and (d) OD and in-phase regime.

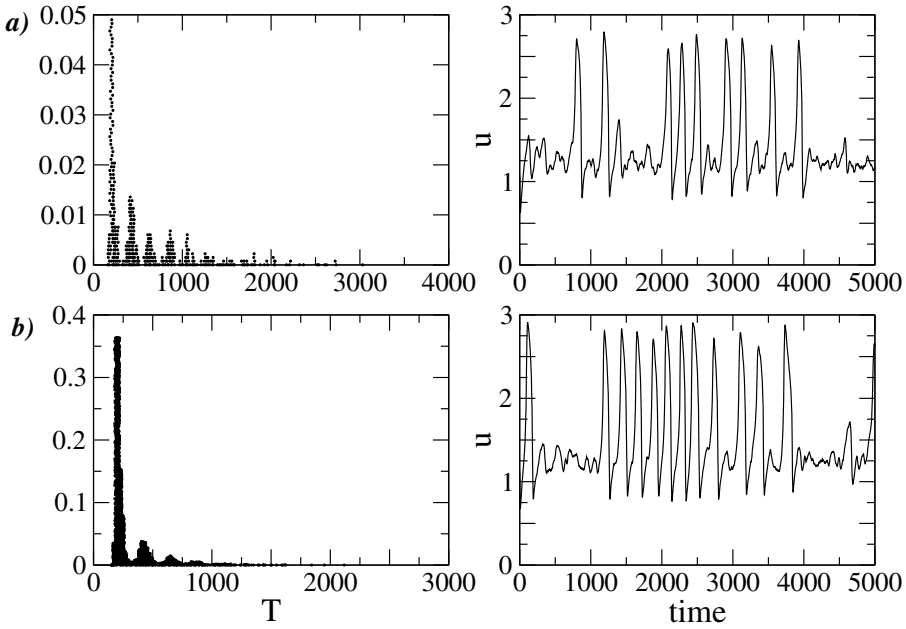

Figure 7. Variability in the ISI (and the corresponding time series) for two nonidentical elements (from an eight-element network): polymodal distributions for (a) $\alpha_1 = 2.928$ and (b) $\alpha_1 = 2.97$. $d = 0.005$, $\sigma_a^2 = 5 \times 10^{-5}$ and other parameters as in figure 2.

environmental conditions, for example, the system is able to switch between different attractors, adapting to the external influence, as manifested in the example with the short noise pulse. Moreover, these mechanisms can be further used as additional control mechanisms for external manipulation of the dynamical behavior of a population of coupled synthetic units.

4. Discussion

The question whether synthetic biology can offer a novel insight into the understanding of cellular mechanisms, and thereof into the controlled production of specific proteins of interest, has become more intriguing and challenging in the past decade. The experimental realizations of single genetic devices and simple modules so far have shown that synthetic biology has the potential to transform our approach to human health, as suggested in Purnick and Weiss (2009). In particular, using engineered synthetic circuits one could establish controlled expression of proteins, which would lead to regulated cellular behavior—an initial step to create new capabilities and effective solutions for drug production and novel therapy treatments, e.g. cells that are programmed to recognize and destroy tumors (Anderson *et al* 2006).

Here, we have discussed the possibility that the construction of synthetic multicellular systems, in contrast to single synthetic units, can provide finely tuned coordinated behavior of the cellular population and, in this manner, provide the possibility for effective control of the dynamical behavior of the system. It has been suggested that intercellular

signaling plays a crucial role in the establishment of cooperative functioning in a population. Thus, we use the intercellular signaling and, in that direction, the dynamical properties of the multicellular system to suggest a mechanism for controlling the dynamical behavior in synthetic genetic networks via a direct manipulation of the (co)existent attractors. In the theoretical analysis presented here, we have shown that the dynamical characteristics of the system could be used as a way of 'fine-tuning' the protein production in interacting cellular populations, if specific cluster distributions in the OD regime are chosen (in terms of a single parameter value or a directed noise pulse). Using the characteristics of the OD solution, one could also provide a way to determine in advance the percentage of cells producing specific protein concentrations in homogeneous and heterogeneous populations. Moreover, the possibility of controlling protein production that we propose here does not depend on the topological structure of the system: we have investigated the particular regulatory mechanism not only in a system of globally coupled synthetic units but also in systems with distinct topology, such as a system of synthetic oscillators, locally coupled on a ring.

Special configurations of cells that would allow for diffusion of AI molecules only between neighboring cells (thereby the local coupling characteristics will be fulfilled) are experimentally not easy to achieve. However, recent advances in so-called rolled-up nanotechnology have led to the realization of the idea to use bioanalytic microsystems for spatial and temporal control of single cells (Huang *et al* 2009). This provides the possibility of confining a precise number of single cells equipped with the corresponding synthetic units in separate nanotubes; thereby spatial and temporal control of the protein production in distinct cells could be achieved.

Additionally, we have investigated the effect that noise has on the dynamical behavior of the system, and the question whether noise can contribute as a regulating factor. We can generalize that a specific control of the dynamics of the system in the presence of noise is possible in two distinct cases: when a short noise pulse is established and when a continuous presence of noise is established. Based on these conclusions, we can speculate that under the influence of changing environmental conditions (which could be interpreted as external noise), for example, the system is capable of switching between different stable attractors, which enhances the fitness of the cellular population under environmental stress, and optimizes the adaptation of the colony by a sensitive adjustment of the protein dynamics.

The new capabilities of synthetic, in this case multicellular, systems presented here serve as an introduction to a new 'wave' of synthetic biology, which will in turn benefit a wide variety of existing fields (such as pharmaceutical and chemical engineering), and will allow the control of dynamical behavior of cells for various purposes. The possible establishment of the control of protein dynamics on a system level will thus enable the design and construction of complex synthetic devices that will ensure functional control of specific cellular mechanisms. In this direction, some of the envisioned applications of the discussed mechanisms include tissue engineering, molecular fabrication of biomaterials and nanostructures, synthesis of pharmaceutical products, and biosensing.

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