Variety of dynamical regimes in a population of coupled synthetic genetic oscillators

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***Abstract*— In this work we review our results on the interplay between stochasticity and intercell coupling in a population of synthetic genetic oscillators with relaxator dynamics. We have shown that control of the coupling strength and noise can effectively change the dynamics of the system, leading to the large variety of different dynamical regimes such as clustering, synchronous and asynchronous oscillations, and noise-induced suppression. Moreover, under certain conditions an optimal amount of noise can lead to increased order in the system, demostrating the effect of coherence resonance.**

In contrast to the previous studies which are mainly focused on synchronized behavior of communicating genetic units, we discuss the question: which mechanisms can be responsible for multirhythmicity in globally coupled genetic units? We have shown that an autoinducer intercell communication system that provides coupling between synthetic genetic oscillators will inherently lead to multirhythmicity and the appearance of several coexisting dynamical regimes. Furthermore, we propose a new mechanism for quantized production time in a network of coupled relaxators, based on the interplay of cell-cell communication and stochasticity. Noteworthy, inhomogeneity can be used to enhance such quantizing effects, while the degree of variability obtained can be controlled using the noise intensity or adequate system parameters.

I. INTRODUCTION

Many fundamental cellular processes are based on genetic regulation programs based on gene-protein interactions. Since the complete structure and functionality of cellular processes remain still mainly unexplained, different mathematical models have been proposed in order to investigate cellular behavior, by using circuit and system theoretic models, including electrical circuits, Boolean and Bayesian networks, differential equations, Petri nets and weight matrices [1], [2] etc. Inspite of the intensive research the investigation of the gene expression dynamics, necessary for chronotherapy of cancer or genetic computations in real time, remains the unsolvable task due to the extreme complexity of gene interactions.

Due to the recent technological advances, the design of synthetic genetic networks has become possible [3], [4] and has been added to the list of theoretical and experimental tools for the study of gene-protein networks. This experimental progress and limited number of genes in host independent synthetic genetic networks has made such networks accessible to quantitative analysis [5], [6]. In certain cases, data obtained from synthetic-biology experiments have been proved consistent with the theoretical predictions of mathematical models, opening the gate for the understanding of gene regulatory networks. So far, the research activities were focused mainly on the design of genetic circuits capable of performing a predefined function. Among the pioneering work is the construction of the toggle switch [4], the repressilator [3], as well as the engineering of several relaxator models [5], [7], [8]. Although started with isolated genetic applets [3], [4], the investigations moved forward in realization of synthetic genetic networks, where separate modules are coupled usually through a type of chemical intercell communication, known as the quorum-sensing mechanism [9], [10], [8].

There are several reasons to investigate synthetic genetic networks: *(i)* the constructed synthetic networks have a rather simple topology with an exactly known structure; *(ii)* the construction of the synthetic genetic networks, using mutually activating or repressing genes (or gene products), enables engineers to evolve biological systems by means of variation and selection for any function they desire, mimicking cell behavior. The design of these synthetic "applets", experimentally realized in simple organisms such as $E.coli$ and $yeast$, is significant not only for the synthesis of artificial biological systems, but also for biotechnological and therapeutic applications [5], [6].

Due to the increased potential of the synthetic genetic networks to offer a well-controlled test bed for the study of the functions of the natural genetic networks or the application possibilities in number of expanding biotechnological fields, the necessity arises for a synthetic circuit capable of producing different rhythm generation mechanisms. This is a very important phenomenon from engineering perspective, since it allows one to gain insight into genetic network function, as well as it offers different possibilities for the construction of new genetic applets.

In this work we review different possibilities to manipulate and control synthetic genetic circuits, providing multifunctional genetic units as an outcome. The role of intercell communication is therefore investigated, to identify mechanisms responsible for presence of multiple rhythms in a synthetic network of coupled genetic units. Furthermore, several possibilities to control the dynamical behavior of the genetic applets are proposed and investigated by means of bifurcation analysis, direct and stochastic simulations. Possible explanations regarding certain naturally occurring mechanism, such as quantized time cycling are also consid-

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ered. Finally, we present an overview of possible applications where these phenomena could be implemented.

II. The Model

Here we review one of the models recently used by us to investigate the role of intercell communication in the appearance of different dynamical regimes. It is important to note that the results reviewed are general and can be observed also in other models of synthetic genetic networks. The underlying genetic circuitry of the model we considered [8] is a hysteresis-based genetic relaxation oscillator, constructed from a toggle switch composed of two genes u and v that inhibit each other, and a quorum sensing mechanism which on one side, provides the transition from trigger to limit cycle in a single cell, whereas by diffusing autoinducer (AI) molecules through the cell membrane, enables the coupling in the network (details are given in [8]). The time evolution of the system is governed by the dimensionless Eqs.:

$$\frac{du_i}{dt} = \alpha_1 f(v_i) - u_i + \alpha_3 h(\omega_i) \quad (1)$$

$$\frac{dv_i}{dt} = \alpha_2 g(u_i) - v_i \quad (2)$$

$$\frac{d\omega_i}{dt} = \varepsilon(\alpha_4 g(u_i) - \omega_i) + 2d(\omega_e - \omega_i) \quad (3)$$

$$\frac{d\omega_e}{dt} = \frac{d_e}{N}\sum_{i=1}^{N}(\omega_i - \omega_e). \quad (4)$$

where N denotes the total number of cells (oscillators), w_i represents the intracellular, and w_e - the extracellular AI concentration (see Fig.1). The mutual influence of the genes is carried out through the functions: $f(v) = \frac{1}{1+v^\beta}$, $g(u) = \frac{1}{1+u^\gamma}$, $h(w) = \frac{w^\eta}{1+w^\eta}$, where β, η and γ are the parameters of the corresponding activatory or inhibitory Hill functions. The dimensionless parameters α_1 and α_2 determine the expression strength of the toggle switch genes, α_3 - the activation due to the AI, and α_4 - the repressing of the AI. The coupling coefficients in the system d and d_e (intracellular and extracellular) depend mainly on the diffusion properties of the membrane [8]. One of the main characteristics of this model is the presence of multiple time-scales, producing relaxation oscillations.

III. Dynamical regimes and controlling mechanisms

Rhythm generation mechanisms are very important for genetic network functions as well as for the design of synthetic genetic circuits. As already mentioned, a significant attention to date has been focused on the synchronization of communicating genetic units, which results in the production of an unified rhythm. On the other hand, multirhythmicity and coexistence of several attractors can be very important for the construction of genetic networks and understanding of evolutionary mechanisms behind the cell differentiation

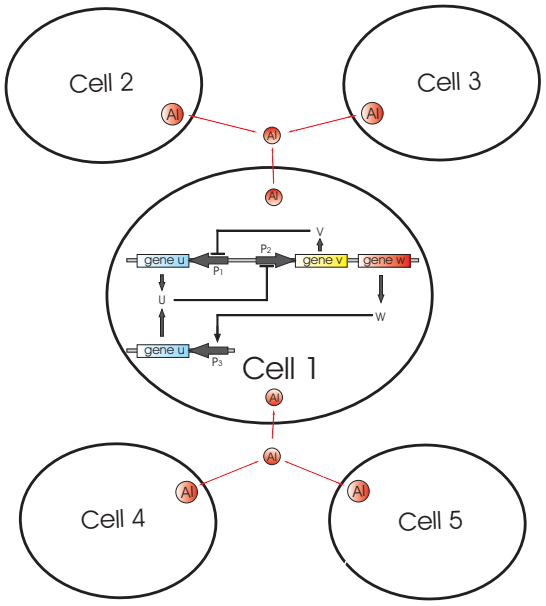

Fig. 1. A scheme of coupled genetic relaxators. The genetic network inside each cell is coupled with another cell by a diffusion of small autoinducer molecules AI, which influence the gene expression.

and genetic clocks functioning. The ability of a genetic unit to produce different dynamical regimes which coexist, also means its improved adaptability: if one of the regimes becomes unprofitable for the cell functioning, the genetic unit can easily switch to some of the other coexistent regimes available. Therefore, we tried to shed a light into the question which mechanisms are responsible for complex dynamical behavior of globally coupled genetic units.

We have identified the complete dynamical structure of the model (Eqn. (1)-(4)) by means of detailed bifurcation analysis, which allowed us to obtain the complete picture of how different solutions are created. Namely, we have shown that an autoinducer intercell communication system which provides inhibitory, phase repulsive coupling between synthetic genetic oscillators in the model described above (Fig.1) will inherently lead to multirhythmicity and the appearance of several coexisting dynamical regimes [11] if the time evolution of the genetic network can be split in two well-separated time scales. The example of the bifurcation diagramm that clearly identifies the coexistence of different dynamical regimes, is shown in the Fig. 2. On this plot the thick solid line denotes the regime of the oscillation death, when all oscillations are silent in two different clusters, and dashed line denotes the stable limit cycle (in-phase regime). It is important to note that bifurcation analysis shows less number of the dynamical regimes which are possible in this system, because the oscillators can be also distributed between the clusters in a different way.

Several different possible modes of organized collective behavior were observed for the first time in networks of coupled synthetic genetic units, such as: anti-phase oscillations, asymmetric oscillations, inhomogeneous oscillatory solution and multiple oscillatory cluster regimes. We have

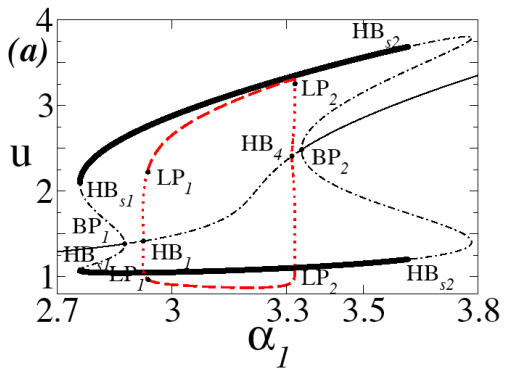

Fig. 2. Illustration of the coexistence of five different states for increased coupling strength $d = 0.3$ and $\varepsilon = 0.05$. Parameters: $\alpha_1 = 3, \alpha_2 = 5, \alpha_3 = 1, \alpha_4 = 4, \beta = \eta = \gamma = 2$ and $d_e = 1$. Coexistence of the oscillation death (OD) and the in-phase oscillatory regime is also shown. Thin solid lines denote stable steady state, thick solid lines - a stable OD regime, dash - dotted lines - unstable steady state, dashed lines - stable limit cycle (in-phase regime) and dotted lines denote unsable limit cycle.

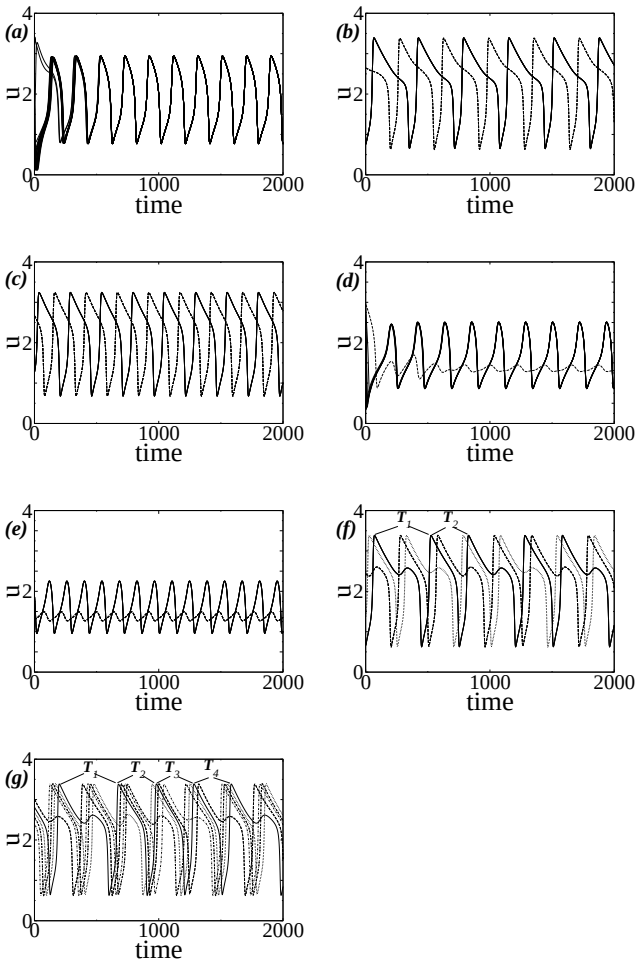

Fig. 3. Different oscillatory clusters for system of $N = 8$ oscillators. **(a):** in-phase oscillations: $\alpha_1 = 3, d = 0.001$; **(b),(c):** anti-phase oscillations with different distributions of the oscillators between clusters: $\alpha_1 = 3.3, d = 0.001$; **(d),(e):** asymmetric solution with different distribution of the oscillators: $\alpha_1 = 2.868, d = 0.001$; **(f):** three oscillatory clusters: $\alpha_1 = 3.3, d = 0.00105$ and **(g):** five oscillatory clusters: $\alpha_1 = 3.3, d = 0.001$. Other parameters are: $\alpha_2 = 1, \alpha_3 = 5, \alpha_3 = 4, \beta = \gamma = \eta = 2, d_e = 1$.

demonstrated also the possibility for different element distribution between clusters, each characterized with different period of oscillations. Furthermore, the multiple oscillatory cluster regimes found could also contain several subcycles, manifested through the generation of different return times in one limit cycle, a novel effect in synthetic circuits (Several examples of the dynamical regimes are presented in Fig. 3). The rhythm generation mechanisms proposed in this work for the first time are of significant importance for various biotechnological applications, since they allow single genetic units to be functional in a wide frequency range.

The obtained general bifurcation structure of the model has predicted more complex behavior of the dynamical system in presence of noise. It is on the other hand known that the biochemical processes of transcription and translation depend on the number of promoter sites and $mRNA$ molecules. These numbers are typically small, and thus cells may experience large fluctuations, which are usually seen as a source of internal noise. Furthermore, noise can also originate externally, in the random variation of one or more of the externally-set control parameters [12]. However, the question of how the cell functions reliably in the presence of noise is still open. Also, the investigation of how the interplay between noise and intercell coupling may lead to qualitative changes in the dynamics of cells has not been pursued at an appropriate level so far. Therefore, we have considered noise and intercell communication as possible mechanism to controll the dynamical state of a multicellular system of synthetic units. For this purpose, we have modified the equation (3) by introducing an additional term $g(w_i)\xi_i(t)$ to model the contribution of random fluctuations. $\xi_i(t)$ is a Gaussian white noise with zero mean and correlation $\langle \xi_i(t)\xi_j(t')\rangle = \sigma_a^2 \delta_{ij}\delta(t-t')$. The multiplicative noise is interpreted according to Stratonovich [13], which is the correct stochastic interpretation for a realistic noise with small temporal autocorrelation [14]. The noise term can incorporate both extrinsic and intrinsic stochastic sources. Physically, this type of noise might be generated by using an external field, e.g. electromagnetic field [5].

We have shown by means of direct and stochastic simulations that the interplay between noise and intercell communication can effectively switch between different dynamical regimes, such as synchronous and asynchronous oscillations and lead to noise-induced suppression of the oscillations in the multicellular system, thus establishing itself as a naturally occurring control parameter for the dynamical regulation in the synthetic genetic ensembles. Moreover, we have shown that for optimal noise intensities, maximal order was observed in the system. In this case the noise-induced jumps in the system were relatively periodic, an resemblance to the coherence resonance effect [20].

The available literature on synthetic genetic oscillators has been focused mainly on the investigation of single genetic units or ensembles of identical elements. However,

in practice it is inevitable that the oscillators are not strictly identical, since cell to cell variation are always present, e.g. small diversity in period lengths of individual cells is always a reality. Therefore, we have drawn a particular consideration also to a network of synthetic genetic relaxation oscillator consisted of nonidentical elements. Such consideration represents almost every experimental situation of interest because it is very difficult to prepare a set of truly identical oscillators in a physical system. The heterogeneity in our investigations is achieved by introducing certain diversity in the α_1 parameter values, not greater than 4% in separate elements. Detailed bifurcation analysis of this case (in preparation) have shown that the multistability and multirhythmicity is inherent in this case as well, where different dynamical regimes are characterized with increased regions of stability. Moreover, new dynamical regimes appear, such as a phase synchronization of order 2:1, previously not reported in the investigations of synthetic genetic networks.

Therefore, complex dynamical behavior can be predicted in the presence of noise, identifying the interplay with heterogeneity and intercell coupling as critical at this point. In order to determine the effective jumps of the oscillators in the system due to noise, we have analyzed statistically the interspike intervals (ISI) and found that the noise in this case contributes to the establishment of variability and well expressed presence of multiple frequencies (see Fig. 4). The cycling is quantized, having uni-, bi- or polymodal solutions. Choosing slightly different α_1 values, one can effectively switch between different mulitpeak distributions, adapting the artificial network to produce the desired frequencies [21].

This approach allowed us to propose a new mechanism for quantized cycling generation, which relies on the interplay between noise and the complex behavior of the dynamical system induced by the specific inhibitory, phase-repulsive intercell coupling and discuss the strategy to control the degree of quantization. The novelty in this approach is also that the polymodality we observe phenomenologically differs from what is commonly reported, e.g. in [15], where the peaks are located as integer multiple spiking with amplitude decay, as a result of the phase preference when external force is applied, whereas the mechanism we propose provides various forms of interspike interval distributions, thus offering a potential explanation why are the modes in the polymodal distributions of generation times observed experimentally not equal to an integer times a quantal period [16].

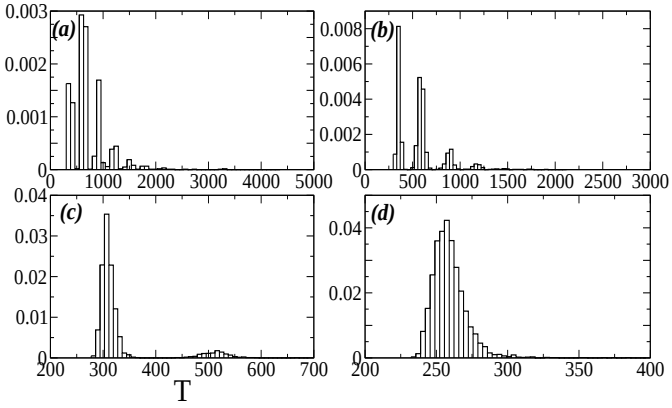

Fig. 4. Variability in the ISI for 4 nonidentical elements (from a 8-elements network): multimodal for **(a):** $\alpha_1 = 3.328$ and **(b):** $\alpha_1 = 3.325$, bimodal for **(c):** $\alpha_1 = 3.31$, and unimodal for **(d):** $\alpha_1 = 3.25$. The noise intensity is $\sigma_a^2 = 5 \cdot 10^{-7}$. Other parameters as in Fig. 4

IV. Conclusions and outlook

To summarize, this work has been focused on detection and analysis of the complete dynamical structure of a model of synthetic genetic networks. Furthermore, the identification of possible mechanism allowing control of gene expression was considered, using naturally occurring control parameters, such as noise, intercell communication and heterogeneity.

The control over gene expression in ensembles of coupled synthetic genetic oscillators opens new approaches in biotechnology, enabling scientists to develop a new era of devices for sensing, computing, drug production, etc. This new approach of investigation, through the construction of synthetic genetic networks implemented in real cells could allow to manipulate biological processes at a genetic level and create more complete models of the behavior of natural systems.

The dynamical richness observed in this particular model can be considered as a significant advantage for a multitude of applications (biosensors, programming genetic units, etc.). It has been reported that multistability is a main mechanism for memory storage and temporal pattern recognition in artificial and natural neural networks [17]. Moreover, the effect of multistability is also used to create an electrically addressable passive device of organic molecules [18] for registration, storage and processing of information. Therefore, it is logical to assume that the ability of the genetic circuits to display multistability opens the possibility for construction of a "new era" computational devices, based on genetic and DNA computing, with data-processing and storage capabilities which would gradually change the direction of computing. If constructed, these new devices will allow more cost-efficient devices that would outweigh present memory units, for example. It is important to point out that these findings were proved to be rather general and model independent, since no specific properties of the investigated system were used. Moreover, we have found similar properties in different relaxator models, such as [9] (work in progress) or in coupled modified repressilators [22]. The strength of quorum sensing can be also used as a bifurcation parameter in this analysis showing that the transition between dynamical regimes can be achieved by variation of the coupling strength between interacting cells. A prerequisite for such a behaviour is the relaxator dynamics of a single oscillator, but, surprisingly, one can also find such a behavior in slightly modified oscillators which initially did not demonstrated relaxator behavior. In this case, the condition for the large variety of dynamical regimes is the breaking of temporal symmetry leading to the appearance of diferent time scales in the

system's dynamics.

In addition, it is very important to note that the presence of different periods we have reported open the possibility for a resonant behavior of the system on multitude frequencies. This result can be important, e.g., for the construction of genetic networks driven by a periodic signal [6] coupled with cell cycle regulation. It also means that different synchronization regions can be obtained for different external frequencies, an effect which can have impact in cancer chronotherapy or cell cycle regulation. We emphasize the generality of these results applicable to other genetic relaxation oscillators (work in preparation), although derived for this particular model of genetic network, since no special properties of the given system were used to obtain the appearance of multistability, multirhythmicity and clustering.

Furthermore, the effects reported might open a new insight into the treatment strategies of the so-called "dynamical diseases" [19]. Considering the temporal dimension of illness, it will be of certain importance whether the therapeutic applications exhibit complex behavior. We hope that due to the simplicity of the genetic motifs we have considered, some of our findings will also contribute to the understanding of naturally produced cell time quantization.

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