COMPLEXITY OF PATTERNS GENERATED BY GENETIC CIRCUITS AND PFAFFIAN FUNCTIONS

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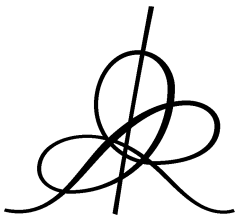

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Complexity of patterns generated by genetic circuits and Pfaffian functions

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Abstract

In this paper the pattern formation ability of some class of genetic circuits is studied. We show that these circuits are capable to generate arbitrary spatio-temporal patterns.

We give upper and lower bounds on the number of genes that are necessary to create a given pattern and study the dependence of this number on the properties of this pattern. Connection between complexity of gene interaction and complexity of patterns arisen is examined. We investigate stochastic stability of patterning algorithm. We explain why these algorithms evolved, and compare the results obtained with experiments.

1 Introduction

This paper deals with special circuits of the neural type playing a key role in contemporary biology, and our results can be applied to the pattern formation problem in biology. Mathematical approaches to this problem started with the seminal paper of A. M. Turing [1]. Turing studied how chemical patterns could emerge from spatially uniform states. His model is a system of two reactions-diffusion equations. Typically in models of such type one reagent diffuses slow, while another one diffuses faster. Turing introduced as well some key notions such as activator, inhibitor and morphogene. Morphogene is a special chemical reagent, which can penetrate in cells and change their states.

Now the existence of such molecules is well known [3],[4]. Moreover, it is proved experimentally that, in multicellular organisms, the state of a cell can depend on gene expression inside this cell and on some signals from environment (electrical, chemical or pressure, [4]).

After [1], similar phenomenological approaches were developed in numerous works (see [5] for a review). Patterns obtained numerically are often similar to patterns actually observed in biology [5]. However, the equations for these models have been selected to be mathematically

tractable and they do not take into account actual experimental genetic information. Moreover, there is no direct evidence for a Turing's patterning any developing organism ([3], p.347).

Recently genetic circuit models were proposed [6]-[9] as more realistic models using the genetic information. These models can be considered as generalizations of the famous Hopfield model of attractor neural network [2].

It is based on two main ideas. The first one is to choose the protein concentrations as state variables for the description of gene regulation. The second one is to use networks similar to neural networks to describe activation or depression of one gene by another. Mathematically these models can have the different forms. It can be described as a system of partial differential equations of a special form [6]-[8], namely

$$\frac{du_i}{dt} = R_i\sigma(\sum_{j=1}^{m} K_{ij}(u_j + \theta_j(x)) - \eta_i) - \lambda_i u_i + d_i\Delta u_i, \tag{1.1}$$

where m is the number of genes included in the circuit, $u_i(x,t)$ the concentration of the i-th protein, λ_i the protein decay rates and d_i the protein diffusion coefficient, the parameters η_i are activation thresholds and σ is so-called sigmoidal function (see below).

We consider (1.1) in some open domain Ω with a regular boundary $\partial\Omega$. If $d_i > 0$ then, in addition to (1.1), we set the standard zero Neumann conditions [41] for u_i on $\partial\Omega$:

$$\frac{\partial u_i}{\partial \mathbf{n}}(x) = 0, \quad x \in \partial\Omega,$$

where $\mathbf{n} = \mathbf{n}(x)$ is the unit vector orthogonal to the boundary $\partial\Omega$ at the point x and directed inward Ω.

If $d_i = 0$ we set no boundary conditions. The initial data equal zero

$$u_i(x,0) \equiv 0, \quad x \in \Omega. \tag{1.2}$$

The real number K_{ij} measures the influence of the j-th gene on the i-th one. The assumption that gene interactions can be expressed by a single real number per pair of genes is a simplification excluding complicated interactions between three, four and more genes. Clearly such interactions can exist, however then the problem becomes mathematically much more complicated. The function σ is a sigmoidal function satisfying the following suppositions

Assumption 1.1 *Suppose σ is continuous strictly monotone increasing function such that*

$$\lim_{z\to-\infty}\sigma(z) = 0, \quad \lim_{z\to\infty}\sigma(z) = 1. \tag{1.3}$$

The well known example is given by $\sigma(z) = \frac{1+\tanh(z)}{2}$. Another example is the Michaelis - Menten sigmoidal function [11]. Notice that the Michaelis-Menten rate, defined as $\sigma(z) = \frac{cz}{1+z}$ for $z \geq 0$ and $\sigma \equiv 0$ for $z \leq 0$, does not satisfy this assumption. However, a part of our results holds in this case.

We assume that θ_i are fixed functions. They give the densities of " maternal genes" that engine pattern growth. (For example, for *Drosophila Melanogaster* the key one of these genes is *bicoid*. The complete number of the maternal genes is about 50, see [3]). Also they can describe concentrations of the substrates involved in patterning. Indeed, we need some food to grow.

Model (1.1) takes into account only three fundamental processes: a) the decay (degradation) of gene products (the term $-\lambda_i u_i$); b) exchange of gene products between cells (the term with Δ) and c) gene regulation and protein synthesis.

Another possible model is a dynamical system with discrete time, for example

$$u_i(x, t+1) = r_i \sigma(\sum_{j=1}^{m} K_{ij} u_j(x,t) + \theta_i(x) - \eta_i) - \lambda_i u_i(x,t) + d_i \Delta u_i(x,t), \qquad (1.4)$$

where $t = 0, 1, 2, ..., T$ and $x \in \Omega$. Numerical procedures solving (1.1) lead to models similar to (1.4). System (1.4) was investigated, for example, in [10], where $\sigma(u) = 1 + sgn(u)$ and $\theta_j(x) \equiv 0$, $\lambda_i = 0, d_i = 0$. In this case the sigmoidal function σ is discontinious and we will not consider this case here. From mathematical point of view, an important adavantage of (1.4) with respect to (1.1) is that, if $d_i = 0$, the Khovanskii [12] results can be applied to this model. In fact, we will see below that (1.4) defines a Pfaffian chain if θ_i and σ are Pffafian.

Besides the mophogenesis problem, systems (1.1) and (1.4) have other interesting applications. For example, consider systems (1.1) where $d_i = 0$ and $\theta_i(x)$ are independent of x but may depend on t. Then system (1.1) reduces to a system of ordinary differential equations. Similar systems have been applied for simulation of networks explaining expression of genes under biochemical and physiological circumstances (see [13] and references in it).

To better understand mathematical formulation of the pattern formation problem and pattern complexity problem for (1.1) and (1.4), let us turn to some biological ideas, concepts and experimental facts.

Let us remind that organisms consist of cells that can be in numerous different states [4].

Neglecting details of the cell structure and following the standard approach [10], we assume that different cell states are obtained as a result of expression of some morphogenes (special genes that can change the cell states).

To explain this situation, we consider, to simplify, the case where we deal with a unique morphogene. For example, let u_m be such a morphogene.

Then cell patterns consist of two kinds of cells: modified and the usual (not modified) ones. If u_m is expressed at x, then we have a modified cell in x, otherwise the cell remains in a usual state.

It can be observed for example in the plant growth. First the embryo grows having an homogeneous structure consisting of identical cells that can divide. When embryo becomes large enough (attains a critical radius, see [14]), cells close to the embryo center change. They cannot divide more. Only cells at the embryo surface stay active and continue to divide. They form so called meristem while centered cells give rise to phloem and xylem [3],[14]. Finally, we see that, in this processus, the plant cells are bistable, and the pattern of cell differentiation is a union of two disjoint sets.

For gene expression that defines sell states, we have a threshold behaviour [3]. We can suppose for instance that the morphogene u_m is expressed if $u_m > c$ and is not expressed otherwise ($u_m < c$). In this case we deal with bistable cells. Here we follow the classical approach, see [1]. This threshold behaviour explains why σ appears in fundamental models (1.1) and (1.4), and allows us to introduce some biologically natural complexity measures, see Sections 4,5 and 6.

Of course models (1.1) and (1.4) are rough simplifications. Actually many other processes should be take into account. In fact, the number involved proteins is of order of many thousands, even a resonable approximation of this process is not known [3]. There is no single universal strategy of patterning ([3], p. 10). Nonetheless it is clear that this rough approximation (1.4) has a connection with actual biology. There are no doubts that threshold mechanisms are important and complicated circuits of interacting proteins exist actually [15]-[17].

To investigate (1.1) and (1.4), the most of the previous works used numerical simulations. For example, the work [8] considered the segmentation in Drosophila, the paper [9] analyzes complicated patterns occurring under a random choice of the matrix K.

The aims of this paper are the following. We concentrate our attention on model (1.4). We

will show that model (1.4) is mathematically tractable, and will give results that are in good accordance with experimental observations. This model allows us to formulate rigorously and analyze some important biological questions such as for example pattern complexity, pattern complexification along evolution processus etc.

First, we will show, in a purely analytical way and without any numerical calculations, that any time sequence of any space patterns can be obtained by genetic circuit (1.5). Second, we examine a connection between the "complexity of a genetic circuit" and the "pattern complexity". Naturally both complexities should be defined in a reasonable way. Third, we are going to investigate stochastic stability of morhogenesis process.

Let us formulate the pattern generation problem mathematically.

Let us fix some function σ satisfying Assumption 1.1. On the contrary, we consider

$N, K_{ij}, \lambda_i, d_i, r_i$ and θ_i as "control" parameters. We denote the set of these parameters by

$$\mathcal{P} = \{m, K_{ij}, \lambda_i, d_i, \eta_i, r_i, \ \ i, j = 1, ..., m \ \}. \tag{1.5}$$

The morphogenesis problem for (1.4) can be described as follows. Given a spatio-temporal pattern and a number $\epsilon > 0$, the problem is to adjust parameters $\mathcal{P}$ of (1.4) such that network (1.4) will generate the given target pattern. The target pattern is defined by a function $z(x,t)$ where $x \in \Omega \subset \mathbf{R}^n, t \in [0,T]$ with the values z from $[0,1]$. Biologically, $z(x,t)$ is a morphogene concentration that defines the state of a "cell" located at x at the moment t.

Pattern generation problem

Let $t_1 > 0$ and $t_1 < T$. Given a function $z(x,t) \in [0,1], x \in \Omega, t \in [0,T]$ and ϵ, to find $m, K_{ij}, \eta_i, d_i, \lambda_i, r_i$ such that the solution of problem (1.4) with initial conditions $u_j = 0$ satisfies

$$\sup_{x,t} |z(x,t) - u_m(x,t)| < \epsilon, \quad x \in \Omega, \quad t = 1, ..., T. \tag{1.6}$$

To evaluate the difference between the target pattern z and the actual pattern u_m we can also use other measures for example

$$S(\mathcal{P}) = \int_0^T (\int_\Omega |z(x,t) - u_m(x,t,\mathcal{P})|^2 dx) dt. \tag{1.7}$$

To obtain the target pattern, we minimize (1.7).

Let us give a biological interpretation of this formulation. Among the genes u_i, we select a special gene, say u_m, that plays a role of the morphogene. The cell states depend on the

expression of this morphogene. Other genes $u_1, u_2, ..., u_{m-1}$ are "hidden genes". They are involved in a cell biochemical machinery, but they do not act directly on the cell states. Such an aproach is in good accordance with experimental facts (see [3],[4]). It reminds classical approaches of neural network theory ([19], [23],[26]) where, similarly, we distinguish "input", "output" neurons and "hidden" ones. To some extent, we justify this separation in the last part of our work.

Let us formulate now main mathematical results, ideas of proofs and give their biological interpretation.

The first result is that problem (1.6) always has a solution, for a sufficiently large network, under some simple conditions on θ_i. The proof is complicated, and for the sake of simplifications, we proceed it in two steps. The first step is to show that the problem can be resolved in one dimensional case. The main idea is a special factorization of matrices K. Earlier, this factorization had many useful applications to various problems such as neural network attractor control, neural optimal control [19]-[22]. To obtain any space structures by (1.4), it is sufficient to have only one monotone non zero function $\theta_i(x)$. Biologically this means that a single maternal gene is sufficient to construct any pattern, even using identical cells, if they have a complicated biochemistry.

To find a patterning algorithm in the two dimensional case, we exploit some facts from molecular and developemental biology [4],[3],[13], [18]. Genes are organised in blocks and their interaction has a modular structure. This idea helps us to construct a patterning algorithm based on the well studied multilayered approximations [23].

Notice that moreover the proof gives us an algorithm that can be efficiently performed numerically. Indeed, it is based on the well studied multilayered approximations [23], [24],[26]. It is well known that they are, in a sense, optimal [25].

These results are stated in Section 2 and 3.

In Sections 4-6 we introduce and apply the different measures of the pattern complexity. The basic biological concepts on gene expression lead, in a natural way, to a mathematical definition of pattern complexity as the number of connectivity components of some sets D defined by the pattern. These sets can be defined in different ways. We consider here two cases. In the first case we define D as a level set,

$$D_{c,t} = \{x : \ z(x,t) = c\}.$$

In the second case

$$D_{c_1,c_2,t} = \{x: \ c_1 \leq z(x,t) \leq c_2\}.$$

Here $0 \leq c \leq 1, 0 \leq c_1 < c_2 \leq 1$. Their biological meaning is that they set thresholds for gene expression (see above).

In the first case, in order to connect the pattern complexity and the circuit parameter, we use estimates following from the fundamental results of Khovanskii [12]. These estimates are independent of the diameter of the domain $\Omega \subset \mathbf{R}^n$ and of the maximum of the absolute values of the entries $|K_{ij}|$. In this case the pattern complexity can be estimated via $(r_\theta + mT + n)$, where parameter r_θ is a complexity of inputs $\theta(x,t)$, the number mT characterizes the complexity of gene interactions.

In the second case we obtain essentially stronger estimates, in a quite elementary inductive way. However, in opposite to the previous ones, these estimates depend on the diameter of the domain Ω and on the maximum of the absolute values of the entries $|K_{ij}|$.

It is not sufficient to have a patterning algorithm; in biology actual algorithms have to be stable. In particular, they must be stable under random noise and sharp changes of ecological conditions. Indeed, ecological catastrophes can eliminate from environment a food that need the growing organisms.

M. Gromov and A. Carbone formulated the following important problem: "Homeostasis of an individual cell cannot be stable for a long time as it would be destroyed by random fluctuations within and without cell. There is no adequate mathematical formalism to express the intuitively clear idea of replicative stability of dynamical systems" ([27], p.40).

In Sections 7-8 we consider the question on the stochastic stability of genetic circuits (1.4). We define stochastic stability of the morhogenesis defined by (1.4) on time interval $[0,T]$ as the probability that the protein density stay inside some fixed bounded domain for all t from $[0,T]$. Notice that such a definition follows standard ideas of the theory of random perturbations of dynamical systems [28]. Tnis probability can be called the survival probability.

As a result of quite elementary estimates we conclude with that **the more is the valency of a node the stabler is the circuit with respect to perturbations in this node**. (The valency of the node is the number of links connecting this node with other ones; in our case the valency of i-th gene is the number of non-zero entries K_{ij}).

This conclusion is in a good accordance with experimental results of [16]. This work in-

vestigated protein networks in 43 microorganisms. It was shown that **the most connected proteins in the cell are the most important for its survival**.

Nonetheless, the survival probability of each circuit of a fixed structure tends to zero as $T \to \infty$ (see the citation from [27] above).

In Section 8 we state an elementary approach to replicator stability. We show that although a fixed isolated circuit is always unstable, a chain of circuits can be stable. In this chain, each circuit is obtained from the previous one by some algorithm modifying the circuit parameter (replication algorithm). This means that the survival probability does not vanish as the time goes to infinity. However, the replication algorithm cannot be arbitrary. We show that, in some cases, only complex replication algorithms lead to "eternal" evolution when the survival probability rests positive for all times.

2 Pattern generation. Main results

We simplify model (1.4) removing the terms describing the protein diffusion and degradation (i.e. we put $\lambda_i = d_i = 0$). We set $\theta_i = \sum_{j=1}^m K_{ij}\theta'_j$ and after the substitution of this relation into (1.5) we omit $'$ to simplify the notation.

As a result, we obtain the following iterative model

$$u_i(x,t+1) = r_i\sigma(\sum_{j=1}^m K_{ij}(u_j(x,t)+\theta_j(x)) - \eta_i), \quad t = 0,1,2,...,T, \quad x \in \Omega \tag{2.1}$$

where

$$u_i(x,0) = 0, \quad x \in \Omega. \tag{2.2}$$

We show that even this simplified model is capable to produce any spatio - temporal patterns. Notice that this system is a particular case of circuits considered in [37]-[40].

Recall that $\theta_j(x)$ are fixed. We denote the set $\{k, k+1, ..., T\}$ by $[k,T]$. To simplify notation, the set $\{1,2,...,T\}$ is denoted by $[T]$.

Problem (1.6) can be reformulated now as follows:

Pattern generation problem

Given a function $z(x,t) \in [0,1], x \in \Omega, t \in [0,T]$ and $\epsilon > 0$, to find m, η_i, r_i and the matrix K such that the iterations $u_i(x,t)$ defined by (2.1)-(2.2) satisfy

$$\sup_{x,t} |z(x,t) - u_m(x,t)| < \epsilon, \quad x \in \Omega, \quad t \in [T]. \tag{2.3}$$

Remark. We cannot satisfy (2.3) for $t = 0$ since initial data are fixed: $u_i \equiv 0$.

First we consider the one-dimensional case. The main result is

Theorem 2.1

Suppose that $\Omega = [0, L] \subset \mathbf{R}$ *and*

$$\theta_i = \theta(x), \quad x \in \Omega, \quad i = 1,$$

$$\theta_i(x) = 0, \quad x \in \Omega, \quad i > 1, \tag{2.4}$$

where θ *is a strictly monotone positive function.*

Then the pattern generation problem has a solution.

Let us turn to the multi-dimensional case. We suppose, for simplicity, $dim\Omega = 2$. The case $dim\Omega > 2$ can be investigated analogously.

First we observe that, in contrast to the one -dimensional case, it is impossible to obtain all patterns with only a single non-zero maternal gene $\theta_1(x, y)$. Indeed, then patterns generated by (2.1) will depend on x, y only through $\theta_1(x, y)$: they have the form $z = f(\theta_1(x, y))$. Thus, at least two different non-zero functions $\theta_i(x, y)$ should be involved in the circuit.

The second main result is

Theorem 2.2

Suppose that $T > 1$, Ω *is an open subset of* $\mathbf{R^2}$ *and*

$$\theta_i = \theta(x), \quad (x, y) \in \Omega, \quad i = 1,$$

$$\theta_i = \tilde{\theta}(y), \quad (x, y) \in \Omega, \quad i = 2,$$

$$\theta_i \equiv 0, \quad (x, y) \in \Omega, \quad i > 2, \tag{2.5}$$

where θ *and* $\tilde{\theta}$ *are strictly monotone positive functions.*

Then, for any given pattern z *and* $\epsilon > 0$, *inequality (2.3) can be satisfied for all* $t = 2, ..., T$.

Let us find conditions on K such that the pattern sequences $u_i(x, t)$ converge as $t \to \infty$.

If we turn to the theory of dynamic systems with the discrete time, such property holds for so-called monotone systems preserving some (partial) order in an appropiate Banach phase space.

For mappings acting in $\mathbf{R}^n$ we can introduce such an order $u < v$ by

$$u < v \text{ if } u_j < v_j \quad \text{for} \quad each\ j. \tag{2.6}$$

Let $u \to F(u)$ be a smooth map. This map F conserves order (2.6) if

$$\frac{\partial F_i}{\partial u_j} > 0, \quad i \neq j. \tag{2.7}$$

In the case (1.4) it holds for matrices K such that $K_{ij} > 0$ for all $i \neq j$.

The theory of monotone dynamical systems have been pioneered by the seminal work of M. Hirsch [43], afterwards developed P.Polacik et al. (for example, [44]), for review see [45]).

Then we can assert that for large t the pattern $u_m(x,t)$ is close to a final pattern. This final pattern is m-th component of a solution of the system

$$u_i(x) = r_i\sigma(\sum_{j=1}^{m} K_{ij}u_j(x) + \theta_i(x) - \eta_i). \tag{2.8}$$

The properties of this pattern can be examined in some cases.

3 Proof of Theorem 2.1 and 2.2

Before start proving we state some basic ideas. First, to simplify the problem, we choose such a matrix K that the morphogene u_m does not influence the other genes $u_i, 1 \leq i < m$.

The reduced matrix $\tilde{K}$ with entries $K_{ij}, i, j = 1, 2, ...m-1$ can be factorized as $K = AB$ where A,B are new matrices (which may be of smaller sizes).

This factorization method was very useful in neural networks, in particular, for problems of associative memory (where one sets $B = A^{tr}$) [2], of optimal control [19], or construction of networks with complicated large time behaviour [19], [21], [22].

We show that such factorization also is useful in our problem and allows us to find a constructive method to resolve problem (2.3).

To simplify the exposition, we split the proof into several steps.

Step 1. First factorization of K. We set $L = 1$ and

$$K_{ij} = \tilde{K}_{ij}, \quad i, j = 1, 2, ..., m-1,$$

$$K_{mj} = \beta_j, \quad K_{jm} = 0, \quad j = 1, 2, ..., m. \tag{3.1}$$

These relations single out the morphogene u_m and simplify the approximation problem. We denote $u_m = U$. We set $r_m = 1$ and $\eta_m = 0$.

Then relations (2.1) take the following form

$$u_i(x, t+1) = r_i \sigma(\sum_{j=1}^{m-1} \tilde{K}_{ij}(u_j(x,t) + \theta_j(x)) - \eta_i), \quad t = [0, T], \quad x \in [0, 1], \tag{3.2}$$

where $i = 1, ..., m-1$ and

$$U(x, t+1) = \sigma(\sum_{j=1}^{m-1} \beta_j(u_j(x,t) + \theta_j(x))), \quad t = 0, 1, 2, ..., T, \quad x \in [0, 1]. \tag{3.3}$$

Since σ is strictly increasing, problem (2.3) can be reformulated as follows: to find m and the numbers $\beta_i, r_i, \tilde{K}_{ij}$ $(i, j = 1, ..., m-1)$ such that iterations (3.2) satisfy

$$|Z(x,t) - \sum_{j=1}^{m-1} \beta_j(u_j(x,t) + \theta_j(x))| < \epsilon_1, \quad t \in [T], \quad x \in [0, 1], \tag{3.4}$$

where

$$Z(x,t) = \sigma^{-1}(z(x,t)), \tag{3.5}$$

σ^{-1} denotes a function inverse to σ, and $\epsilon_1 = \epsilon_1(\epsilon)$ is a small positive number.

Step 2. Second factorization.

Let us introduce the linear subspace E_m

$$E_m = \{U : \quad U = \sum_{j=1}^{m-1} \beta_j u_j(x,t) \ \} \tag{3.6}$$

of functions defined on the direct product $\Pi_T = [0, 1] \times [T]$.

Reformulation (3.4) of the problem shows that in order to complete the proof, it is enough to prove that this subspace E_m is dense in the space of all continuous functions (with sup-norm) in Π_T. To end this, we use the approach based on the simplest factorization of $\tilde{K}_{ij}$. Namely, we set

$$\tilde{K}_{ij} = a b_j, \quad i, j = 1, ..., m-1, \tag{3.7}$$

where a and b_i are new unknown coefficients. We set $r_i = 1$ for all i.

Let us introduce the quantity

$$q(x,t) = \sum_{j=1}^{m-1} b_j u_j(x,t). \tag{3.8}$$

Multiplying i-th equation (3.2) by b_i and summing up the equations obtained over i from 1 to $m-1$, we have

$$q(x,t+1) = \Phi(q(x,t) + \bar{\theta}(x)), \quad q(x,0) = 0, \tag{3.9}$$

where the function Φ is defined by

$$\Phi(v) = \sum_{i=1}^{m-1} b_i \sigma(av - \eta_i), \tag{3.10}$$

and $\bar{\theta}(x) = b_1\theta_1(x)$, $b_1 > 0$.

If the functions $q(x,t)$ are found, one can obtain u:

$$u_i(x,t+1) = \sigma(a(q(x,t) + \bar{\theta}(x)) - \eta_i), \quad t \in [0,T]. \tag{3.11}$$

To simplify the notation, we introduce the new variable $Q = q + \bar{\theta}$. Relations (3.10) and (3.11) then take the following form

$$Q(x,t+1) - \bar{\theta}(x) = \Phi(Q(x,t)), \quad Q(x,0) = \bar{\theta}(x). \tag{3.12}$$

and

$$u_i(x,t+1) = \sigma(aQ(x,t) - \eta_i). \tag{3.13}$$

Now approximation inequality (3.4) can be rewritten as

$$|Z(x,t) - \sum_{j=1}^{m-1} \beta_j \sigma(aQ(x,t) - \eta_j)| < \epsilon, \quad t \in [T], \quad x \in [0,1]. \tag{3.14}$$

The key idea can be described as follows. Suppose for different instants of time t the images $R_t(\Omega)$ of the mappings $R_t : x \to Q(x,t)$ are disjoint. That is

$$R_t(\Omega) \cap R_{t'}(\Omega) = \emptyset, \quad (t \neq t'). \tag{3.15}$$

Moreover, let us assume that for each t the function $Q(x,t)$ is strictly monotone in x.

Then approximation (3.14) exists. Indeed, in this case we can consider Q as an independent variable instead of (x,t). Let us define the set

$$\mathcal{R} = \cup_{t=0}^{t=T} R_t(\Omega).$$

We can introduce a new function $\bar{Z}(P)$ by

$$\bar{Z}(P) \equiv Z(x(P), t(P)) \quad x \in [0,1], \quad t \in [T], \tag{3.16}$$

where a new variable P ranges over the set $\mathcal{R}$ and where $x(P), t(P)$ are defined by

$$Q(x(P), t(P)) = P, \quad P \in \mathcal{R}.$$

Consider the following inequality

$$|\bar{Z}(Q) - \sum_{j=1}^{m-1} \beta_j \sigma(aQ - \eta_j)| < \epsilon, \quad Q \in \mathcal{R}, \tag{3.17}$$

where $\mathcal{R}$ is a finite union of some intervals in $\mathbf{R}$, and $\bar{Z}$ is given continuous function. It is clear that this inequality is equivalent to (3.14).

We satisfy (3.17) in a certain larger set $\mathcal{R}'$, $\mathcal{R} \subset \mathcal{R}'$. We take this set $\mathcal{R}'$ as the convex hull $Conv\mathcal{R}$. Therefore, without loss of generality, we can assume that $\mathcal{R}'$ is some interval: $\mathcal{R}' = [Q_-, Q_+]$.

We set $\tilde{\eta}_i = Q_- + \frac{(i-1)(Q_+ - Q_-)}{m-2}$ where $i = 1, ..., m-1$. Let us denote

$$\delta_a^i(Q) = \sigma(a(Q - \tilde{\eta}_i)) - \sigma(a(Q - \tilde{\eta}_{i+1})) \tag{3.18}$$

and put $\xi_i = (\tilde{\eta}_i + \tilde{\eta}_{i+1})/2$. We observe now that for any continuous $\tilde{Z}(Q)$

$$\sup_{Q \in \mathcal{R}'} |\tilde{Z}(Q) - \sum_{i=1}^{m-1} \tilde{Z}(\xi_i) \delta_a^i(Q)| \to 0 \tag{3.19}$$

as $a, m \to \infty$. Relation (3.19) gives us, for large m, a, an approximation satisfying (3.17). In fact, it is clear that the unknown coefficients β_i, η_i can be expressed via $m, \tilde{Z}(\xi_i)$ and $\tilde{\eta}_i$.

Thus, to complete the proof, it is sufficient to show that, for η_i and a defined above, there is a choice of b_i such that the functions $Q(x,t)$ satisfy relation (3.15) and being strictly monotone for each t in x.

We notice that

$$Q(x,0) = \bar{\theta}(x), \quad Q(x,1) = \bar{\theta}(x) + \Phi(\bar{\theta}(x)),$$
$$Q(x,2) = \Phi(\bar{\theta}(x) + \Phi(\bar{\theta}(x))) + \bar{\theta}(x), ... \tag{3.20}$$

Let us forget temporarily that Φ is a sum of σ -functions.

It is easy to find a function $\Phi(v)$ such that iterations (3.20) fulfil (3.15) for $t \in [0, T]$. In fact, let the interval $[\theta_-, \theta_+]$ be the image of $[0,1]$ under $\bar{\theta}(x)$. We set $\Phi(v) = \lambda v$ where λ is a positive coefficient such that $\lambda\theta_- > \theta_+$. We observe now that n-th iteration $Q(x,n)$ is

defined by $Q = (\lambda^n + \lambda^{n-1} + ... + 1)\bar{\theta}(x)$. The image of this function is the interval $I_n = [(\lambda^n + \lambda^{n-1} + ... + 1)\bar{\theta}_-,\ (\lambda^n + \lambda^{n-1} + ... + 1)\bar{\theta}_+]$. These intervals are disjoint since

$$(\lambda^n + \lambda^{n-1} + ... + 1)\bar{\theta}_- > (\lambda^{n-1} + \lambda^{n-2}... + 1)\bar{\theta}_+$$

due to the condition $\lambda\theta_- > \theta_+$.

Having a smooth Φ with the property (3.15) and sufficiently large a and m, we can find a sum of the form $\Phi^b = \sum_{i=1}^{m-1} b_i\sigma(a(v - \eta_i))$ close enough to λv by a choice of b_i. We notice that if property (3.15) holds for Φ, it also holds for sufficiently small (in supremum norm) perturbations of Φ. It completes the proof.

Proof of Theorem 2.2.

We approximate 2-dimensional patterns using some special two 1 -dimensional patterns which can be obtained by the Theorem 2.1. To complete it, we split the genetic circuit into some blocks.

We consider a circuit of a special structure. It consists of four gene groups:

a) genes $u_1,\ u_2, ..., u_{m_1}$, b) genes $\tilde{u}_1,\ \tilde{u}_2, ..., \tilde{u}_{m_1}$, c) $v_1, v_2, ..., v_p$ and d) u_m. Here $m = 2m_1 + p + 1$.

The dynamics is organized as follows. Let

$$u_i(x, t+1) = \sigma(\sum_{j=1}^{m_1} K_{ij}(u_j(x,t) + \theta_j(x)) - \eta_i), \quad t \in [0, T], \quad x \in \Omega, \tag{3.21}$$

where $\theta_j = \theta(x)$ for $j = 1$ and it is 0 otherwise.

Let

$$\tilde{u}_i(y, t+1) = \sigma(\sum_{j=1}^{m_1} \tilde{K}_{ij}(u_j(y,t) + \tilde{\theta}_j(y)) - \eta_i), \quad t \in [0, T], \quad x \in \Omega, \tag{3.22}$$

where $\theta_j = \tilde{\theta}(y)$ for $j = 1$ and it is 0 otherwise.

These equalities entail that there are no interactions between $\tilde{u}$ and u-genes. For v_k we set

$$v_k(x, y, t+1) = \sigma(\alpha_k u_{m_1}(x,t) + \tilde{\alpha}_k\tilde{u}_{m_1}(y,t) - h_k). \tag{3.23}$$

At last, for u_m we set

$$u_m(x, t+1) = \sigma(\sum_{k=1}^{p} b_k v_k(x,t)). \tag{3.24}$$

The approximation problem can be reformulated now as

$$|\tilde{Z}(x,y,t) - \sum_{k=1}^{p} b_k v_k(x,y,t)| < \epsilon, \quad t = 2,3,...,T. \tag{3.25}$$

where $\tilde{Z} = \sigma^{-1}(Z)$. Due to Theorem 2.1, iterations (3.21) and (3.22) can approximate any sequences (along t) of u_{m_1} and $\tilde{u}_{m_1}$ patterns (depending on the parameter choice).

In particular, they can generate the following sequences

$$u_{m_1}(x,t) = X_t(x) + \delta_t(x), \quad \tilde{u}_{m_1}(y,t) = Y_t(y) + \tilde{\delta}_t(y), \tag{3.26}$$

where $\delta(x,t)$ and $\tilde{\delta}(y,t)$ are small corrections such that $|\delta| + |\tilde{\delta}| < \epsilon_0$. Suppose that $x \in [x_0, x_1]$ and $y \in [y_0, y_1]$ as the point (x,y) ranges over Ω.

We assume that X_t and Y_t satisfy the following properties. Let X_t be strictly monotone, smooth and the images $X_t[x_0, x_1]$ be disjoint for different t:

$$X_{t_1}[x_0, x_1] \cap X_{t_2}[x_0, x_1] = \emptyset, \quad t_1 \neq t_2, \tag{3.27}$$

for $t_i \in [0, T]$. The functions Y_t also are strictly monotone, smooth and give disjoint images:

$$Y_{t_1}[y_0, y_1] \cap Y_{t_2}[y_0, y_1] = \emptyset, \quad t_1 \neq t_2. \tag{3.28}$$

Due to the well known results on multi-layered networks [23]-[26] the following assertion holds: for any continuous $Z(X,Y)$ defined on a compact domain, and any $\epsilon > 0$ we can find a sigmoidal approximation

$$Z^{\sigma}(X,Y) = \sum_{k=1}^{p} b_k \sigma(\alpha_k X + \tilde{\alpha}_k Y - h_k) \tag{3.29}$$

satisfying

$$|Z(X,Y) - Z^{\sigma}(X,Y)| < \epsilon/4, \tag{3.30}$$

for appropriate $p, b_k, \alpha_k, \tilde{\alpha}_k$ and h_k. Notice that iterations $\sigma^{-1}(u_m)$ have the following form

$$\sigma^{-1}(u_m(x,y,t)) = \sum_{k=1}^{p} b_k \sigma(\alpha_k(X_t(x) + \delta_t(x)) + \tilde{\alpha}_k(Y_t(y) + \tilde{\delta}_t(y)) - h_k). \tag{3.31}$$

Inequality (3.25) takes, in this notation, the following form

$$|\tilde{Z}(x,y,t) - \sum_{k=1}^{p} b_k \sigma(\alpha_k(X_t(x) + \delta_t(x)) + \tilde{\alpha}_k(Y_t(y) + \tilde{\delta}_t(y)) - h_k)| < \epsilon. \tag{3.32}$$

We can choose a sufficiently small ϵ_0 such that the inequality

$$|\tilde{Z}(x,y,t) - \sum_{k=1}^{p} b_k \sigma(\alpha_k X_t(x) + \tilde{\alpha}_k Y_t(y) - h_k)| < \epsilon/2 \tag{3.33}$$

implies (3.32). Therefore, it rests to satisfy (3.33). We make it by (3.30) and arguments from the proof of Theorem 2.1.

Relations (3.27) and (3.28) imply that there is an one-to-one mapping connecting points X, Y from the union of the images $X_t[x_0, x_1] \times Y_t[y_0, y_1]$ and points (x, y, t). Thus, (3.33) can be obtained from (3.29) and (3.30). The Theorem is proved.

4 Complexity of a pattern and complexity of a network

In this section we consider the following problem. Suppose we observe some sequence of patterns $z(x,t),\ z \in \Omega,\ t \in [0,T]$. We would like to estimate the number of genes required to create this sequence.

To resolve this problem, we can use different characteristics of pattern complexity. In this paper, we employ the following three quantities: $C_1(z(\cdot,\cdot), c)$, $C_2(z(\cdot,\cdot), c_1, c_2)$, $E(z(\cdot,\cdot))$. They are functions of the discrete time t.

The quantity C_1 is the number of connected components of the set

$$D_{c,t} = \{x :\ z(x,t) = c\}. \tag{4.1}$$

To define C_2, let us consider a set $D_{c_1,c_2,t}$ depending on two parameters c_1, c_2 and t. Namely, let us define

$$D_{c_1,c_2,t} = \{x :\ c_1 \le z(x,t) \le c_2\}. \tag{4.2}$$

Then C_2 is the number of such connected components of this set on which the function z attains both values c_1 and c_2. Thereby, the image under z of any of these components coincides with the interval $[c_1, c_2]$.

Both complexity measures are discrete, whereas E is a continuous quantity, defined by

$$E = \int_\Omega |\nabla z|^2 dx. \tag{4.3}$$

Let us discuss now the biological sense of C_1, C_2 and E and relations between them.

Organisms consist of cells and these cells can be in different states. Following the ideas stated in Introduction, we assume that different cell states appear as a result of expression of different morphogenes. We consider here the case of one morphogene. Let u_m be such a morphogene.

Then we can study structures consisting of two kinds of cells: modified and the usual ones. If u_m is expressed at x then we have here a modified cell, otherwise the cell remains in a usual state.

Following the theshold approach (see Introduction) suppose that the morphogene u_m is expressed if $u_m > c$ and it is not expressed in the opposite case ($u_m \leq c$). In this case we arrive, as a natural measure of complexity, to the quantity C_1.

Possibly however, that a more realistic biologically approach is using of C_2 where we assume that u_m is expressed if $u_m > c_2$ and it is not expressed if $u_m < c_1$. In the case $c_1 < u_m < c_2$ we deal with an intermediate (transient) state.

Thus both C_1 and C_2 relate to the number of transitions between cells of different types.

Notice that using Sard' theorem, we can choose c, c_1, c_2 in definitions (4.1) and (4.2) such that at least locally the boundaries of the connected components will be smooth submanifolds of Ω of the codimension 1. In particular, if Ω is an interval, these components will be isolated points.

Example. For periodical layered structure C_1=C_2= number of layers (for appropriate c, c_1, c_2).

The third measure, the quantity E, can be interpreted as a mean value of "oscillations" of z.

The results for C_1 and C_2 are quite different. To estimate m through C_1 we use so-called Pfaffian chains [12], under some additional assumptions on σ. It allows us to obtain rough estimates of C_1 by Khovanski's results. Estimates of C_2 and E can be derived in a simpler way and appear to be essentially better.

Up to now, nobody knows whether the Khovanskii bounds can be improved. The key difference between estimates of C_1 on the one hand, and C_2, E on the other is that the estimates of C_2 and E depend on the diameter $diam(\Omega)$ of domain Ω whereas the ones of C_1 are independent of this diameter.

5 An estimate of m via C_1

Let us introduce the key notion of a Pfaffian chain [12], [29].

Definition. A Pfaffian chain of the length r and degree $d \geq 1$ is a sequence of real analytic functions $f_1(x), f_2(x), ...f_T(x)$ in $\mathbf{R}^n$ with the following property: every f_j, $1 \leq j \leq T$ satisfies a Pfaffian equation

$$\frac{\partial f_j}{\partial x_k} = g_{kj}(x, f_1(x), ..., f_j(x)), \tag{5.1}$$

where g_{kj} are polynomials of degrees $\leq d$. Then T is called the length and d the degree of the Pfaffian chain.

Pffafian functions are well studied. They enjoy the following properties: the sum and the product of two Pfaffian functions f_1 and f_2 of lengths r_i and degrees d_i are again Pffafian functions of length $r_1 + r_2$ and degree $d_1 + d_2$ for both the sum and the product. Superpositions of Pfaffian fucntions also are Pfaffian (see [29] for details).

Consider some elementary examples. The exponent $\exp(ax)$, $x \in \mathbf{R}$ is a Pfaffian function of length 1 and degree 2. More generally, any real analytic function $f(z)$, $z \in \mathbf{R}$ satisfying an equation

$$\frac{df}{dz} = P(z, f) \tag{5.2}$$

is a Pfaffian of degree $degP$. We observe thus that many classical sigmoidal functions are Pfaffian. For example, $f = (1 + \exp(z))^{-1}$ satisfies (5.2) with $P = f^2 - f$, for the Michaelis -Menten function relation (5.2) holds with $P = k^{-1}(1 - f)^2$.

Superposition $\sigma(\exp(ax))$ also is a Pfaffian, etc.

The results of this section hold under the following additional assumption.

Assumption 5.1. Suppose σ satisfies (5.2) for some $P = P(z, f)$ and θ_i also are Pfaffian.

Let us show first that under this assumption chain (2.1) can be considered as a Pfaffian chain. Let us introduce complexity of chain (2.1) as the tuple of integers

$$Comp = \{\ m,\ T,\ r_\theta,\ d_\theta,\ degP\}, \tag{5.3}$$

where r_θ is the sum of the lengths of Pfaffian chains for θ_i, d_θ is the maximum of the degrees of Pfaffian chains determining θ_i, $degP$ is the degree of the polynomial from (5.2) that defines σ.

Let us introduce a notation: $u_i^t(x) = u_i(x, t)$ are functions obtained at t-th step.

Using induction, let us consider now the functions u_i^1. By differentiating, one has

$$\frac{\partial u_i^1}{\partial x_l} = r_i \sigma'(\sum_{1 \le j \le m} K_{ij}\theta_j - \eta_i)(\sum_{1 \le j \le m} K_{ij} \frac{\partial \theta_j(x)}{\partial x_l}).$$

Consequently by assumption 5.1 one obtains

$$\frac{\partial u_i^1}{\partial x_l} = P(\sum_{1 \le j \le m} K_{ij}\theta_j - \eta_i, \frac{u_i^1}{r_i})(\sum_{1 \le j \le m} K_{ij} P_{j,l}(x, v_1^j, v_2^j, ..., \theta_j)), \tag{5.4}$$

where $P_{j,l}$ are appropriate polynomials, v_k^j are functions of chains determining θ_j. Thus, u_i^1 and θ_j form a chain of the degree $d_\theta + degP$ and the length $r_\theta + m$. Repeating these calculations, we conclude that $u_i^t, u_i^{t-1}, ...\theta_i$ form a chain of the degree $d_\theta + tdegP$ and the length $r_t = r_\theta + tm$.

Now the complexity of the pattern $u_m(x,T)$ can be estimated applying known results ([12], see also [29], Proposition A4).

Theorem 5.1. *The number C_1 of the connected components of the output pattern $u_m(x,T)$ can be bounded from above by*

$$C_1 < 2^{(r_\theta + Tm)^2} (d_\theta + TdegP)^{O(r_\theta + Tm + n)}. \tag{5.5}$$

Thus given C_1 we can bound from below $R = r_\theta + Tm$ roughly as $(\log_2 C_1)^{1/2}$, provided that $\log(degP), \log(d_\theta), n^{1/2}$ are less than $r_\theta + Tm$. The quantity R can be interpreted as a "complexity" of gene circuit (2.1).

This estimate does not look optimal but in general case up to now there exist no methods that could improve it.

However, if we consider rational σ, for example Michaelis-Menten case, then this estimate can be improved.

Recall that matrices K_{ij} which actually meet in biological applications are "sparse" i.e. each gene interacts only with few other genes. To describe this situation, we introduce the following characteristics: the valency V of the circuit. For each i we define V_i as the number of entries K_{ij} such that $K_{ij} \neq 0$. Then V is the maximum of V_i over i.

We first consider u_m^T as a function of variables $\theta_1, \theta_2, ..., \theta_s$. (We suppose after permuting subscripts that u_m^T actually depends only on s functions θ_i among $\theta_1, \theta_2, ..., \theta_m$).

Finally, for Michaelis -Menten circuit we consider the following set as a complexity of the circuit

$$Comp_M = \{\ m, s,\ T,\ r_\theta,\ d_\theta\}. \tag{5.6}$$

We will show now that, under suitable suppositions, the final pattern u_m^T is a rational function in $\theta_1, \theta_2, ..., \theta_s$ and calculate the degrees of the numerator and the denominator of this function. It allows us to evaluate C_1 through $Comp_M$.

Assumption 5.2 *Suppose that chain u_i^t consists of strictly positive functions.*

(This assumptions is natural from chemical or biological point of view and means that concentrations of u^i do not vanish).

Again we apply an inductive procedure. Let us consider $u_i^1(\theta)$. We see that

$$u_i^1 = \frac{\sum_j K_{ij}\theta_j}{1 + \sum_j K_{ij}\theta_j} = R_i^1/Q_i^1,$$

where R^1 and Q^1 are polynomials in θ_k of degree 1. At the second step, we have

$$u_i^2 = \frac{\sum_j K_{ij}(R_j^1/Q_j^1 + \theta_j)}{1 + \sum_j K_{ij}(R_j^1/Q_j^1 + \theta_j)}. \tag{5.8}$$

By elementary transformations we find from (5.8) that

$$u_i^2 = R_i^2/Q_i^2,$$

where $degR_i^2,\ degQ_i^2 \leq V + 1$.

Continuing this procedure for the final pattern we find

$$u_i^T = R_i^T/Q_i^T, \quad degR_i^T, degQ_i^T \leq (V+1)^{T-1}. \tag{5.9}$$

Applying the Khovanski's bound [12] to the polynomials R_m^T we conclude with the following proposition:

Proposition 5.1 *Under assumption 5.2, the complexity C_1 of the pattern $u_m(x,T)$ of a Michaelis-Menton circuit does not exceed*

$$2^{r_\theta^2}(V^T + d_\theta)^{r_\theta + n}. \tag{5.10}$$

6 Estimates of E and C_2

The estimates of the previous section were independent of $\max_{i,j} |K_{ij}|$ and the diameter $diam\Omega$. Throughout this section we assume that the domain Ω is open and topologically trivial (contractable). In this section the bounds on E and C_2 are stronger than the ones on C_1 from the

previous section, but hold under the conditions that

$$\max_{i,j} |K_{ij}| \leq K_*, \quad diam\ \Omega = \delta > 0. \tag{6.1}$$

Other parameters which we use in our estimates are V (the circuit valency defined above) and

$$\rho = sup_{i,k} |\frac{\partial \theta_i}{\partial x_k}|. \tag{6.2}$$

Moreover, we assume in this section that $\sigma \in C^1$, posesses a continuous derivative and

$$\sup \sigma'(z) = C_\sigma. \tag{6.3}$$

Now we can estimate ∇u_i^t inductively. Indeed, denote $\sup_{i,x} |\nabla u_i^t| = \mu^t$. Then

$$\mu^{t+1} \leq C_\sigma (V K_* \mu^t + \rho), \quad t = 0, 1, ..., \tag{6.4}$$

where $\mu^0 = 0$. Therefore,

$$\mu^t \leq \rho C_\sigma \frac{(C_\sigma V K_*)^t - 1}{C_\sigma V K_* - 1} \tag{6.5}$$

if $a = C_\sigma V K_* \neq 1$ and

$$\mu^t \leq t \rho C_\sigma, \tag{6.6}$$

if $a = 1$. We can suppose without any loss of generality that $a \neq 1$.

It is obvious then

$$E(u_m^t) < c\delta^n (\rho C_\sigma \frac{C_\sigma V K_*)^t - 1}{C_\sigma V K_* - 1})^2, \quad n = dim \Omega. \tag{6.7}$$

Now we proceed to an estimate of C_2 and begin with the one-dimensional case. The inequality $C_2 > k$ where k is an integer, entails that there are two points x_1, x_2 such that

$$|x_1 - x_2| < \delta/k, \quad u_m^t(x_1) = c_1, \quad u_m^t(x_2) = c_2. \tag{6.8}$$

Thus there is a point ξ such that

$$|\frac{du_m^t}{dx}(\xi)| > \frac{(c_2 - c_1) C_2}{\delta}. \tag{6.9}$$

But by (6.5) we obtain then

Proposition 6.1 *If Ω is an interval of length δ, then the following estimate of the pattern complexity via the circuit complexity holds:*

$$C_2 < diam\Omega(c_2 - c_1)^{-1}\rho C_\sigma \frac{(C_\sigma V K_*)^t - 1}{C_\sigma V K_* - 1}. \tag{6.10}$$

It gives us the required estimate. Let us notice that an analogue of this estimate also holds for continious model (1.1). Its deduction is similar, and we leave it to a reader.

Let us turn now to the case $n = dim\Omega > 1$.

Theorem 6.2. *If Ω is a topologically trivial domain with the smooth boundary, we have for generic c_1 and c_2 that*

$$C_2(c_1, c_2, u_m^T) < const\ mes\Omega\ (\rho C_\sigma \frac{(C_\sigma V K_*)^T - 1}{C_\sigma V K_* - 1})^n. \tag{6.11}$$

We start with an elementary assertion: if each connected component contains a ball of a radius r, then the number of connected components

$$C_2 < const\ mes\Omega\ r^{-n}, \tag{6.12}$$

where the factor *const* depends on n.

Now, to prove Theorem, we are going to estimate r.

First we, using Sard's Theorem, choose c_1, c_2 such that they are regular values of a smooth function u_m^T.

Consider a connected component D_k of the set defined by (4.2). Then the boundary ∂D_k is a union of two disjoint smooth manifolds B_i of the codimension 1, $B_i = \{x : u_m^T(x) = c_i\}, i = 1, 2$, herein we employ the theorem on a regular value, see [42]. Since the boundaries are compact, there are two points $x^1 \in B_1, x^2 \in B_2$ such that

$$dist(x^1, x^2) = \inf_{x \in B_1,\ y \in B_2} dist(x, y). \tag{6.13}$$

Let us set $2r = dist(x^1, x^2)$ and show that the open ball $\mathcal{B}$ which have the interval $[x^1, x^2]$ with the endpoints x^1, x^2 as a diameter is contained in D_k.

Indeed, we have just two possibilities: either $\mathcal{B}$ lies completely in D_k or completely outside of D_k. Otherwise, $\mathcal{B}$ would contain some points of the boundary ∂D_k, for example a point z where $u_m^T(z) = c_1$. But then $dist(z, x^2) < r$ that gives us the contradiction with (6.13).

Let us check now that the second possibility ($\mathcal{B}$ is outside of D_k) also leads to a contradiction.

Let us denote by W the unique connected component of B_1 which contains the point $x^1 \in W$. Since W is a smooth submanifold of the codimension 1, due to the Alexander's duality [47] the complement $\Omega \setminus W$ consists of two connected components U_0, U_1 (taking into account the topological triviality of Ω). Then D_k lies completely in one of U_0, U_1, let $D_k \subset U_0$ for definiteness. The interval $(x^1, x^2]$ (with deleted endpoint x^1) does not intersect W (due to (6.13)), therefore this interval is contained completely either in U_0 or in U_1. On the other hand, the point $x^2 \in D_k \subset U_0$, hence the whole interval $(x^1, x_2] \subset U_0$.

For a small enough ball $\mathcal{B}_{x^1}(e)$ centered at x^1 the complement $\mathcal{B}_{x^1}(e) \setminus W$ has two connected components (again we make use of that W being a smooth submanifold of the codimension 1 and a connected component of the boundary of D_k). One of these two components coincides with $\mathcal{B}_{x^1}(e) \cap D_k$ and another one with $\mathcal{B}_{x^1}(e) \setminus \overline{D_k}$. This partition is the same as the partition of $\mathcal{B}_{x^1}(e) \setminus W$ into two connected components $\mathcal{B}_{x^1}(e) \cap U_0$ and $\mathcal{B}_{x^1}(e) \cap U_1$. Because we have $D_k \subset U_0$ we conclude that $\mathcal{B}_{x^1}(e) \cap D_k = \mathcal{B}_{x^1}(e) \cap U_0$. Therefore, a suitable beginning $(x^1, x^3] \subset (x^1, x^2]$ of the interval $(x^1, x^2]$ is contained in $\mathcal{B}_{x^1}(e) \cap D_k$ (see the previous paragraph). Taking into account that the open interval (x^1, x^2) does not intersect the boundary of D_k thanks to (6.13), this implies finally that $(x^1, x^2) \subset D_k$ which is a contradiction with that $\mathcal{B}$ is outside of D_k.

To conclude the proof, it is sufficient now to estimate r. Using the Lagrange theorem, we obtain

$$c_2 - c_1 = 2r|(\mathbf{n} \cdot \nabla u_m)|,$$

where $\mathbf{n}$ is a unit vector directed along the diameter $[x^1, x^2]$. This relation entails

$$r^{-n} \leq C \sup |\nabla u_m|^n.$$

Applying estimates (6.5) and (6.12), we obtain (6.11).

Notice that the complexities C_1 and C_2 are stable under small perturbations.

Lemma 6.1 *For generic c and c_i the complexities C_1, C_2 of the pattern $u_m(x,t)$ are conserved under small smooth perturbations of patterns: the complexities of the pattern u_m coincide with the corresponding complexities of $u_m + \tilde{z}(x)$ if $|\tilde{z}_{C^1}| < \epsilon$ and ϵ is small enough.*

Proof. Consider the case C_2. The connected components are disjoint. Since they are compact, the distances d_k between these components are positive. If c_1, c_2 are regular values of u_m, their boundaries are smooth submanifolds of the codimension 1. If ϵ is sufficiently small, the perturbation of these level submanifolds are small, due to the regularity of the values c_i.

Thus, since $\inf d_k > 0$, the perturbed connected components rest disjoint.

An interesting particular case is given by the Michaelis - Menten dynamics. Suppose all the entries K_{ij} are positive. Then the patterns converge. Final patterns $u_i(x)$ satisfy (see section 2)

$$u_i(1 + \sum_{j=1}^{m} K_{ij}u_j + \theta_i) = \sum_{j=1}^{m} K_{ij}u_j + \theta_i. \tag{6.14}$$

From Khovanski's bounds we get for the solutions of (6.14) the bounds on their complexities

$$C_1, C_2 < 2^{r_\theta^2}(m + d_\theta)^{r_\theta + n}.$$

7 Stochastic Stability

For applications the important meaning has the problem of pattern stability under perturbations of external parameters. The problem on the structure of protein circuits and in particular on the form of such structure which can support homeostasis stability under fluctuations attracts a great attention of biologists (see [15]-[17], [46]).

We prove here some estimates on stability under noise leading to important biological consequences. Moreover, we develop an approach to the replicator stability answering to the question of M. Gromov and A. Carbone, cited in the Introduction.

Consider a perturbed problem (2.1):

$$u_i(x, t+1) = \sigma(\sum_{j=1}^{m} K_{ij}u_j(x, t) + h_i(x) - \xi_i(t)), \tag{7.1}$$

where $h_i = \theta_i - \eta_i$. Here $\xi_i(t)$ are some random processes with the discrete time. We assume that they are independent for different i. The random quantities $\xi_i(t)$ can be distributed, for example, according to gaussian laws $\mathcal{N}(e_i, \kappa_i)$ with average e_i and deviations $\kappa_i > 0$. Different choices of the values ξ_i may correspond to different 'ecological conditions'. We introduce two functions

$$Prob(\xi_i(t) > a, \text{ for some } t \in [T_1, T_2]) = \Phi_i(a, T_1, T_2) \tag{7.2}$$

and

$$Prob(\xi_i(t) < a, \text{ for all } t \in [T_1, T_2]) = \Psi_i(a, T_1, T_2). \tag{7.3}$$

It is clear that $1-\Phi_i=\Psi_i$. The following assumption plays an important role in what follows. Suppose

$$\Psi_i(a,T_1,T_2)>0, \quad (T_2>T_1), \quad \Psi_i(a,T_1,T_2)\to 0 \ as \ T_2\to\infty. \tag{7.4}$$

This means roughly speaking that ξ_k can take any large values with non-zero probabilities. This assumption holds for the gaussian probability distribution. It is clear that $\Phi_i(a,T_1,T_2)$ are increasing functions of T_2 for any fixed a while $\Psi_i(a,T_1,T_2)$ are decreasing.

Suppose that an "organism" (a protein circuit (7.1)) "survives" (supports homeostasis) if the concentrations u_i stay at some closed domain Π in the u -phase space.

We assume $\sigma(z)\in(0,1)$. Notice that then

$$u_i(x,t)\in(0,1). \tag{7.5}$$

We suppose that Π is contained inside the cube $[0,1]^m$.

As a mesure of the stochastic stability of homeostasis, we consider the probability

$$P(\mathcal{P},\Pi,\Omega,T_1,T_2)=Prob\{u_i(x,t)\in\Pi \text{ for each } x\in\Omega, \text{ and } t\in[T_1,T_2]\}. \tag{7.6}$$

This probability depends on the circuit parameters $\mathcal{P}$, the homeostasis domain Π and Ω. We will name it the survival probability on the time interval $[T_1,T_2]$ and denote by $P(T_1,T_2)$ omitting the dependence on $\mathcal{P}$, Π and Ω. Such definition of the stability is standard in the theory of dynamical system [28]. However, one can introduce other important measures of stability for example with respect to random eliminations of some proteins or vanishing of some entries of the matrix K. This stability is under great attention in recent works connected with the random graph theory (see the review [48]). We will not consider such stability here.

We estimate the stability via the following parameters: the valency, the maximum $|K_*|$ of absolute values of the entries K_{ij}, the maximum b of $|\theta_i(x)|$ and some parameter N_{key} that we introduce below. It is important to take into account the valency since it is well known that biological circuits are not completely connected: for each fixed node i we have a valency $V_i<m$: only V_i of the entries K_{ij} are non-zero.

To introduce N_{key}, let us observe that

$$\inf_{u\in\Pi} u_i=W_i\geq 0. \tag{7.7}$$

Denote $U_i=\sigma^{-1}(W_i)$. Some W_i and U_i could be positive. The corresponding indices $i_1,...,i_s\in[m]$ we name key indices and the corresponding proteins we name the key ones. In fact, if

$W_i > 0$, this means that the organism cannot survive if the concentration of i -th protein is small enough at some points. The morphogenes should be the key proteins. The number s of the key proteins is denoted by N_{key}. We denote I the set of key indices corresponding to the key proteins.

Consider (7.1). Let us take some key index $i \in I$. We have the following simple inequality

$$\sum_{j=1}^{m} K_{ij} u_j(x,t) + \theta_i - \xi_i \le S_i = V_i K_* + b - \xi_i. \tag{7.8}$$

Thus, if

$$\xi_i(t) > V_i K_* + b - U_i, \tag{7.9}$$

the concentration $u_i(x, t+1)$ is less than the critical value W_i. Moreover, if at least one $u_i(x,t)$ is less than W_i at some point x, the state $u(x,t)$ is outside of this domain Π. Thus, we have

$$Prob\{u(x,t) \in \Pi,\ t \in [T_1+1, T_2],\ x \in \Omega\} < \prod_{i \in I} \Psi_i(V_i K_* + b - U_i, T_1, T_2 - 1). \tag{7.10}$$

Therefore, we have proved

Proposition 7.1 *The survival probability satisfies*

$$P(T_1, T_2) < \prod_{i \in I} \Psi_i(V_i K_* + b - U_i, T_1 - 1, T_2 - 1) = P_+(T_1, T_2). \tag{7.11}$$

This estimate yields interesting biological consequences. Notice that the function P_+ is a monotone increasing function of the valency. It is decreasing as the number N_{key} of the key proteins increases. Moreover, the sharper is the sigmoidal function σ, the larger is P_+. The most interesting conclusion is the following. **The more valency of the node the circuit is stabler with respect to perturbations in this node**.

It is in an accordance with experimental results of the work [16]. They show that **the most connected proteins in the cell are the most important for its survival**.

Moreover, we notice that all circuits are unstable, more precisely stochastically unstable as the time T goes to infinity. In fact, assumption (7.4) and estimate (7.11) imply that

$$P(0,T) \to 0 \quad as\ T \to \infty. \tag{7.12}$$

Then there arises a natural question: how to stabilize the circuits. We will consider it in the next section.

8 Replicator Stability

We show in this section that a periodic renovation (replication) of the circuit parameters $\mathcal{P}$ can transform stochastically unstable systems to the stable ones. However, this system transformation must satisfy some important restrictions. We can consider these transformations as an algorithm of "evolution". The key question is about the algorithm properties providing the stability.

We consider circuits (7.1) under the assumptions of the previous section.

We also suppose that $\xi_i(t)$ are identical independent random processes in a certain sense, homogeneous in time. More precisely, let

$$\Phi_i(a, T_1, T_2) = \Phi_i(a, 0, T_2 - T_1). \tag{8.1}$$

Consider possible schemes of renovation. It can be described as follows.

Each T_r time steps we change the circuit parameters $\mathcal{P}$ following some rule. For example, each T_L time steps we can add to the network a new link, and each T_n steps, we include a new node (protein). Here T_n and T_L are some positive integers. We can also use more sophisticated schemes, for example add new nodes with many links. In the case of graphs, differents schems of graph evolution are studied by many works, see for a review [48].

Let us calculate the survival probability. Let $P_n = P(\mathcal{P}_n, [nT_r, nT_r + T_r])$ be the probability to survive within the time interval $[nT_r, (n+1)T_r]$. Here $\mathcal{P}_n$ are the circuit parameters in this time interval.

The probability to survive on the interval $(0, \infty)$ is then the infinite product

$$P_\infty = P_1 P_2 P_3 ... = \prod_{n \in \mathbf{N}} P_n.$$

Consequently, the quantity P_∞ is non-zero if the series $\log P_1 + \log P_2 + ... + \log P_n + ...$ converges. We have obtained thus the following assertion.

Proposition 8.1. *The survival probability P_T remains positive as $T \to \infty$ if and only if the series*

$$\log P(\mathcal{P}_0, [0, T_r]) + \log P(\mathcal{P}_1, [T_r, 2T_r]) + ... + \log P(\mathcal{P}_n, [nT_r, (n+1)T_r]) + \tag{8.2}$$

converges. If this series disconverges to $-\infty$, the survival probability tends to zero as time tends to infinity.

Propositions 7.1 and 8.1 yield an elementary consequence that gives us a sufficient condition for stochastic stability in infinite time. Notice that it is more precisely to say about the stochastic stability of the pair (circuit, replication algorithm) rather than about the stochastic stability of circuits.

Proposition 8.2. *The survival probability P_T tends to zero as $T \to \infty$ if the series*

$$\sum_{i\in I} \log(\Psi(V_i^0 K_* + b - U_i, 0, T_r)) + \sum_{i\in I} \log \Psi(V_i^1 K_* + b - U_i, T_r, 2T_r)) + ...$$

$$+ \sum_{i\in I} \log \Psi(V_i^n K_* + b - U_i, nT_r, (n+1)T_r)) + ... \tag{8.3}$$

disconverges. Here V_i^n are the valencies at the n-th renovation step.

To prove it, let us notice that, due to Proposition 7.1, $-\log P(T_1, T_2) > -\sum_{i\in I} \log \Psi(V_i K_* + b - U_i, T_1 - 1, T_2 - 1)$.

Although these results look quite elementary, nonetheless they allow to analyze the different evolution algorithms and lead to interesting biological consequences.

Consider some examples.

Example 1.

Let us suppose that all the proteins are key ones. Suppose that their stability is a priori bounded:

$$\inf_i U_i > \bar{U} > 0. \tag{8.4}$$

Biologically, this means that the protein stability is a priori bounded during evolution. Let us suppose that the renovation algorithm is, in a certain sense, simple. This means that the renovation procedure either adds to the circuit a node (protein), with a link, or only a link connecting some existing nodes.

Then such evolution is always unstable. To prove it, let us consider series (8.3). First we notice that if the protein number m is bounded as $T \to \infty$ then the circuit chain is finite and it is unstable due to (7.4) and (7.10). Thus, we can assume that $m \to \infty$ as $T \to \infty$. Then series (8.3) contains infinitely many of the terms that are negative and less than

$$\mu_n = \log \Psi(K_* + b - \bar{U}, nT_r - 1, (n+1)T_r - 1), \tag{8.5}$$

since the valency of new proteins is $V = 1$. Due to the time homogeneity hypothesis (8.1) we observe that $\mu_n = \mu$ is independent of n. Also μ is non zero number, according to assumptions

(7.4). Thus series (8.3) disconverges. We obtain analogous negative results even if each new protein enters the circuit with many links but under the condition that the valency of this new protein stays a priori bounded.

Example 2.

Let us suppose that only a part of all the proteins are key ones. Suppose that (8.3) holds. Assume that the renovation procedure adds to the circuit a node (protein), with a link, and this link always is not a key protein. (Therefore, the number of the key proteins conserves).

Then such evolution can be stable or unstable depending on the properties of the processes ξ_k. To see it, let us consider series (8.3). For large n we can use the asymptotics

$$\log \Psi(V_i^n K_* + b - U_i, nT_r, (n+1)T_r)) = \log(1 - \Phi(V_i^n K_* + b - U_i, nT_r, (n+1)T_r)) \approx$$

$$\approx \Phi(V_i^n K_* + b - U_i, nT_r, (n+1)T_r). \tag{8.4}$$

Let us consider the case of gaussian random processes, with a constant deviation $\kappa_i(t) = \kappa$ and the zero means. Then, for example, if V_i^n grows as $O(\log n)$ as $n \to \infty$, this series converges.

Hovewer, if we, from time to time, add key proteins and their number grows unboundedly, the evolution becomes unstable.

Finally, we can perform a stable evolution (i.e. to have $\lim P_T > 0$ as $T \to \infty$) only if the renovation algorithm is complicated itself. Namely, the key protein enters for the circuit together with many links, and the number of new links increases unboundedly.

Notice that the algorithm described in the proof of Theorem 2.2 satisfies this property of the complexity. In fact, let us remind that this algorithm works as follows. Roughly speaking we take a matrice K consisting of two independent blocks. We introduce a set of new proteins (nodes) linked with previously existing proteins by a number of links. At last, we put in the protein system a new key protein (morphogene) having a number of connections with the previous proteins.

Another interesting consequence of this approach and results of Sections 2-3 is a possibility, in principle, to define an "evolutionary" Kolmogorov's type complexity for patterns generated by a chain of evolving circuits. (For the definition of the Kolmogorov complexity see [30]-[32]). However, we will not consider this question here.

Other probability approaches to this evolution problem can be found in [30], [33], [35], [36], however they do not concern the pattern formation problem.

9 Conclusion

To conclude, let us summarize the main biological results. In this paper we have considered a simple model of genetic circuits controlling morhogenesis process. We have shown that genetic circuits are capable to produce any sequences of any space patterns. The algorithm making it is efficient numerically. Moreover, this algorithm is based on a modular structure of the circuit. It is consistent with the contemporary ideas in molecular biology [18]. Roughly speaking, if a genetic circuit "knows" a target pattern, then the circuit constructs it in an efficient way.

Moreover, we have introduced different measures of pattern complexity and found estimates with the aid of the machinery due to Khovanskii [12], of the pattern complexity via the genetic circuit parameters. This approach is new for such applications.

At last we have studied the stochastic stability of the circuits under random fluctuations of its environment.

We have shown that each isolated circuit is unstable under noise, in a sense, that it will be inevitably destroyed by fluctuations after large time. The obtained estimate of the survival probability is consistent with the experimental data [16]: the larger is the mean circuit valency the stabler is the circuit, besides that, the sharper is the sigmoid, also the stabler is the circuit.

Although each fixed circuit is unstable as time goes to infinity, a chain of the modified circuits can be stable. Here we suppose that there is an algorithm that modifies the circuit parameters from time to time. We have obtained some rough estimate of the chain stability.

We have proved, under some conditions, that, to perform an eternal evolution (where chances to survive stay positive as time tends to infinity), the replication algorithm have to be complex. For example, a new protein should enter for the circuit together with many links. To some extent, it can be considered as an explanation of existence and complexity of the replication mechanism.

References

[1] A. M. Turing, The chemical basis of morphogenesis, Phil. Trans. Roy. Sco. B, **237**, 37 -72 (1952).

[2] J. J. Hopfield, *Neural networks and physical systems with emergent collective computational abilities*, Proc. of Natl. Acad. USA, **79** (1982), 2554 – 2558.

[3] L. Wolpert, R. Beddington, T. Jessell, P. Lawrence, E. Meyerowitz and J. Smith, *Principles of Development* (Oxford University Press, 2002).

[4] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson *Molecular Biology of the Cell* (3rd ed. 1994), Garland Publishing, Inc. New York 1994.

[5] H. Meinhardt, *in Mathematical Models for Biological Pattern Formation*, edited by P. K. Maini, H. G. Othmer, IMA Volumes in Mathematics and its Applications, **121**, (Springer, New York, Berlin, Heidelberg, 2000).

[6] E. Mjolness,D. H. Sharp and J. Reinitz, A connectionist Model of Development, J. Theor. Biol. (1991) **152** 429-453.

[7] J. Reinitz and D. H. Sharp, Mechanism of formation of eve stripes Mechanisms of Developments (1995),**49**, 133-158.

[8] D. H. Sharp and J. Reinitz, Prediction of mutant expression patterns using gene circuits, BioSystems, (1998) **47**, 79-90

[9] I. Salazar-Ciudad, J. Garcia-Fernadez and R.V. Solé, "Gene Networks Capable of Pattern Formation: From Induction to Reaction-Diffusion," in *Journal of theoretical Biology*, **205** (2000), 587-603.

[10] L. Mendoza and E. R. Alvarez-Buylla, Dynamics of Genetic Regulatory Networks for *Arabodopsis thaliana* Flower Morphogenesis, J. Theor. Biol. (1998), **193**, 307-319

[11] A. L. Lehninger, D. L. Nelson and M. M. Cox, Principles of Biochemstry, 2nd. ed. New York: Worth, 1993.

[12] A. Khovanskii, Fewnomials, Translations of Mathem. Monographs, Amer. Math. Soc., **88**, (1991)

[13] M.K. S. Yeung, J. Tegner, and J. J. Collins, Reverse engineering gene networks using singular decomposition and robust regression, PNAS, (2002), **99**, 6163-6168.

[14] V.M. Buren, V. A. Nedorezov, Cell mechanisms of wheat development, Ufa, State University, 2002.

[15] H. Jeong, B. Tombor, R. Albert, Z. N. Otvai and A.L. Barabási, Nature (London), (2000), **407**, 651-654.

[16] H. Jeong, S. P. Mason, A.L. Barabási and Z. N. Otvai, Nature (London), (2000), **411**, 41-42.

[17] D. Eisenberg, E. Marcotte, I. Xenerios and T. O. Yeates Nature (London), (2000), **405**, 823-826.

[18] L. H. Hartwell, J. J. Hopfield, S. Leibler and A W Murray, From molecular to modular cell biology, Nature, (1999),**402**, C47 -C52.

[19] Funahashi K and Nakamura Y, 1993 Approximation of Dynamical Systems by Continuous Time Recurrent Neural Networks, *Neural Networks*, **6**, 801- 806.

[20] S. A. Vakulenko, *A system of coupled oscillators can have arbitrary prescribed attractors*, J. Phys A: Math. Gen, **27**, (1994) 2335 -2349

[21] Vakulenko, S. A. and Gordon, P. V. Neural networks with prescribed large time behaviour. J. Phys. A. Math. Gen. (1998), **31**, 9555–9570.

[22] S. A. Vakulenko, Dissipative systems generating any structurally stable chaos, Advances in Diff. Equations, 2000,**5**, 1139-1178

[23] R. Hecht-Nielsen, *Kolmogorov's mapping neural network existence theorem*, IEEE International Conference on Neural Networks, San Diego: SOS Printing (1987), 11-14.

[24] G. Cybenko, *Approximation by superpositions of a sigmoidal function*, Mathematics of Control, Signals and System, (1989), **2**, 303-314.

[25] A. Barron, *Universal Approximation Bounds for superpositions of a sigmoidal functions*, IEEE Trans. on Inf. theory, (1993),**39**, 930-945.

[26] K. Hornik, M. Stinchcombe M and H. White, *Multilayer feedforward networks are universal approximators*, Neural Networks, (1989), **2**, 359-366.

[27] M. Gromov, A. Carbone Mathematical slices of molecular biology, (2001), Preprint IHES/M/01/03

[28] Ventsel, A. D. and Freidlin, M. I.: Random Perturbations of Dynamic systems (New York: Springer) 1984

[29] D. Grigoriev and N. Vorobjov, Complexity Lower Bounds for Computation Trees with Elementary Transcendental Functions Gates, Theoret.Comput.Sci., (1996), **157**, 185-214.

[30] M. Li, P. Vitanyi, Introduction to Kolmogorov complexity and its applications, Springer, 1997.

[31] A. N. Kolmogorov, Logical basis for information theory and probability theory, IEEE Trans. Inform. Theory 14: 5 (1968) 662-664.

[32] A. Zvonkin, L. Levin, The complexity of finite objects and the algorithmic concepts of information and randomness, Russian Math. Surveys, **25** (1970), 83-124..

[33] P. Pudlak, Complexity Theory and Genetics: Computational Power of Crossing Over, *Information and Computation*, **171** 201 -223 (2001)

[34] Clote P, and Bachofen, R Evolution as a computational engine, in "Proc. Computer Science Logis. Aarhus Denmark Lecture Notes in Computer Science, Vol. 1414, pp. 35-55, Springer -Verlag, Berlin (1997)

[35] Rabinovich Y, Quadratic Dynamical Systems and Theoretical Aspects of Genetic Algorithms Ph D. Thesis, Hebrew University, Jerusalem.

[36] T. Jansen and I. Wegener, Royal road functions - Where crossing over is provably essential GECCO 2001, Genetic and Evolution Computation Conference, San Francisco, CA, pp. 375-382.

[37] D. Grigoriev, Deviation theorems for solutions of linear ordinary differential equations and applications to parallel complexity of sigmoids. — St.Petersburg Math. J., (1995), **6**, N 1, p. 89-106.

[38] D. Grigoriev, Deviation theorems for pfaffian sigmoids. — St.Petersburg Math. J., (1995), **6**, N 1, p. 107-112.

[39] D.Grigoriev, Approximation and complexity: Liouvillean type theorems for linear differential equations on an interval. – Foundations of Computational Mathematics, (2001), **1**, p. 289–295.

[40] D.Grigoriev, Approximation and complexity II: iterated integration. – Foundations of Computational Mathematics, 2002,**2**, p. 295–304

[41] J. D. Murray, *Mathematical Biology*, Springer, New York, Berlin, Heidelberg, 1993.

[42] M. W. Hirsch, Differential topology, New- York, Heidelberg, Berlin, 1976.

[43] M. W. Hirsch, *Stability and convergence in strongly monotone dynamical systems*, *J. Reine. Angew. Math.* (1988),**383**, 1- 58

[44] Polácik P.and Terescák T, Convergence to Cycles as a Typical Asymptotic Behaviour in Smooth Strongly Monotone Dicrete -Time Dynamical Systems, Arch. Rat. Mech. Anal. 116 (1991) 339–360.

[45] H. L. Smith and H. R. Thieme, *Convergence for strongly order preserving semiflows*, SIAM J. Math. Anal., **22**, (1991), 1081 -1101.

[46] D. J. Lockhart and E. A. Winzeler, Nature (London), (2000) **405** 827-836.

[47] W. S. Massey, A basic course in algebraic topology, Springer, 1991.

[48] R. Albert and A. L. Barabási, Statistical mechanics of complex networks, Rev. Modern Physics, (2002),**74**, 47-97