Decision Making in an Intracellular Genetic Classifier

L. Abrego[1], A. Zaikin[1,2,3]*

[1] Department of Mathematics, University College London, London, UK

[2] Institute for Women's Health, University College London, London, UK

[3] Lobachevsky State University of Nizhniy Novgorod, Nizhniy Novgorod, Russia

Abstract. A model for an intracellular genetic classifier is introduced and studied to investigate how cellular decision making will function under the stochastic conditions. In particular, this provides a basis to investigate whether a binary classification under the effects of intrinsic noise is still possible. More precisely, a mathematical model of a genetic classifier is derived using a standard approach using Hill functions and its dynamical properties are explored. Classification mechanism is studied considering the effects of low copy number of mRNA and proteins in terms of the degree of cooperativity, inputs and transcription rates. It is shown that the intrinsic noise blurs the separation line between the classification classes, but the influence of stochasticity is qualitatively different for the case of monostable or bistable dynamics. Finally, potential applications are discussed.

Keywords and phrases: binary classification, decision making, intrinsic noise, perceptron, intelligence

Mathematics Subject Classification: 92B20, 92B05

1. Introduction

Classification of external stimuli is fundamental for several biological processes in multicellular organisms [1]. In particular, development of cellular systems can be regarded as a sequence of decisions involving a response of these stimuli in which cells, by means of regulation of specific genes, choose among distinct cell-states leading to a diverse set of biological behaviors. Examples of particular interest in which cell decision-making is involved include differentiation [2], apoptosis [3], pattern formation [4] and survival [5]. Thus, it should come as no surprise that understanding this process has attracted much attention to explain cellular phenomena of different complexity.

This cellular decision-making includes intracellular processes such as regulation of genetic networks within the cell, which involves chemical reactions among its biological constituents. These genetic networks, in turn, serve to transduct and process the information of extracellular signalling. As a result, the determination of cellular fate is an integral process involving its internal genetic architecture and the exploitation of information from external influence. One key aspect is that cell decisions are performed under the influence of fluctuating conditions due to external and internal factors [6–8]. One one hand, extrinsic noise is consequence of the difference between cells in multicellular organisms, which affects their

---

*Corresponding author. E-mail: `alexey.zaikin@ucl.ac.uk`

gene expression. On the other hand, intrinsic noise comes from the low copy number of the biochemical components in the cell, leading them to fluctuate in concentration. Both sources of noise are inherent in all cellular processes. It is in this context that cells must take an appropriate response from the amount of information obtained. In this sense, if noise is sufficiently considerable such that diminishes cellular functionality, this will decrease the efficiency of the whole decision process.

It is also observed from previous research that noise may be a fundamental source to improve the response of living organisms in which a decision process is involved [5,9]. Still, investigating the underlying principles of stochastic decision in cell fates is of foremost importance in modern biology. In particular, a current challenge is to understand the decision-making under well-defined conditions. Approaches from synthetic biology have been used to gain insight into this issue by constructing proof-of-principle systems. Recently, it was shown the constructive role of noise for this decision process in a synthetic genetic classifier circuit [10, 11]. The model is motivated to function as a genetic perceptron with the ability of discriminating external stimuli while providing one out of two possible states. In this sense, this system is able to make intelligent decisions by performing binary classification from several external stimuli. Applications of this system were discussed, where it is speculated that neurons may have an intracellular network on a genetic level based on the principle of artificial intelligence [12].

More precisely, this genetic classifier scheme considers two external inputs which are attributed to the expression of two genes in terms of the concentrations of their respective proteins. In turn, the inputs are weighted by the strength of their transcriptional regulation coefficients following a classification according to a well-defined rule. In this way, the output value will depend on the state of the whole classifier at any time. In the work proposed in [10] the influence of noise in linear classification was studied when the threshold of classification is spoiled. Their results show that additive noise following a normal distribution and discriminated according to a linear classification leads to stochastic resonance in the accuracy of classification. This resonance effect is reproduced in other study considering a dynamical setting where noisy input signals represent additive noise [11]. In both investigations the models do not consider the involved chemical reactions and the sources of noise derived from these ones. We believe this may play an important role for the classification process and provide a more plausible picture for the synthetic biological system. Thus, in the present work we extend the study of this genetic classifier to account for the underlying gene expression mechanisms and their stochastic nature. In this sense, we will have an insight into the robustness of this genetic perceptron under the effects of noise. This investigation may have potential applications to understanding decision-making, particularly, in neural networks [12].

We define and address the problem of classification under the effects of intrinsic noise. Accordingly, in this paper a stochastic model for the scheme of a genetic perceptron proposed in [11] is developed. Since the model is now based on the Hill type transcriptional regulation, derived from the underlying chemical reactions, the influence of intrinsic noise is studied using standard methods to perform stochastic simulations. More precisely, by considering biochemical interactions through Hill functions, we show that in contrast with the linear classification proposed in [10], the system is able to perform nonlinear separation. In particular, linear classification is observed for the case of noncooperative binding and is associated with monostable dynamics; whereas for the positive cooperativity it is shown that the system exhibits bistability.

2. Model

The model considered here has been studied in order to understand binary classification under the influence of intrinsic noise. The genetic scheme is shown in figure 1 following [10, 11] and based on the Kaneko model [13]. The genetic network consists of 5 genes organized as follows: expression from genes A and B sets the inputs in the system, while gene C, D and E the threshold unit. In particular, genes D and E have a toggle switch scheme which maintains a state ON-OFF or OFF-ON, where expression of gene D sets the output. Finally, gene C has a constant basal activity, which represses activity of gene D and activates it for gene E. In this way, C regulates the threshold in the classification process.
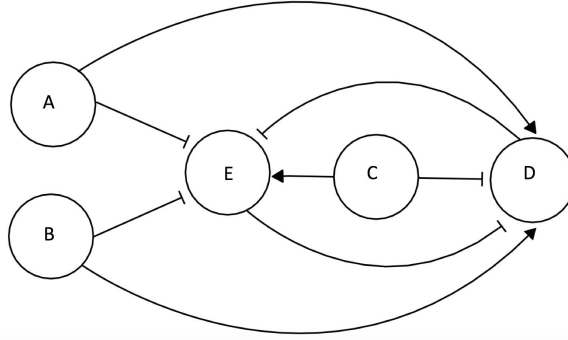
FIGURE 1. Design of binary classifier. The scheme consists of five genes. Gene expression from A and B set the input for the threshold unit E-C-D. The threshold is composed of a toggle switch E-D which is influenced, i.e., biased, by expression of gene C.

Gene expression for the system shown in figure 1 is formulated using the framework provided by the Hill functions to describe inhibitory and activatory processes [14]. A set of coupled ordinary differential equations expressed in (1) is obtained, which provides the time evolution of concentration of mRNA m_i and protein p_i for each gene $i = A, B, C, D, E$.

On the one hand, the mRNA dynamics is governed by degradation and transcription for all five genes. Importantly, since genes A, B and C are not regulated by any other genes, their dynamics are decoupled from the set of equations. In contrast, the dynamics in m_D considers the transcriptional activation by inputs A and B and inhibition by gene E, while the dynamics in m_E reflects the transcriptional activation by gene C and inhibition by gene D. These considerations, derived from first principles, are observed in the particular expression for genes D and E. On the other hand, protein dynamics is governed by degradation and translation with equal rate assumed identical for the five genes.

The model expressed in (1) is dimensionless in such a way time is measured in units of mRNA lifetime by assuming it equal for all genes; protein concentrations are in units of their Michaelis constant also considered equal for all the genes. The degree of interaction between transcription activators and inhibitors and binding sites in each gene is considered through cooperativity coefficient $n > 0$. In addition, α_i is the dimensionless transcription rate in the absence of repressor and a_i is the basal activity, where $i = A, B, C$. Basal activity is assumed different of zero in order to avoid a decay in a long-run for inputs and gene C, which regulates the threshold permanently. For the protein dynamics we have that coefficient β is the ratio between mRNA with respect to protein lifetimes and mRNA concentration is scaled to its translation efficiency (proteins produced per mRNA, assumed equal for all the genes). It is important to point out that it is still not clear what the effective biochemical constants are for such a model.

$$\frac{dm_A}{dt} = a_A - m_A \qquad\qquad \frac{dp_A}{dt} = \beta(m_A - p_A)$$

$$\frac{dm_B}{dt} = a_B - m_B \qquad\qquad \frac{dp_B}{dt} = \beta(m_B - p_B)$$

$$\frac{dm_C}{dt} = a_C - m_C \qquad\qquad \frac{dp_C}{dt} = \beta(m_C - p_C) \qquad (1)$$

$$\frac{dm_D}{dt} = \frac{(\alpha_A p_A^n + \alpha_B p_B^n)}{(1 + p_A{}^n)(1 + p_B^n)(1 + p_C^n)(1 + p_E^n)} - m_D \qquad\qquad \frac{dp_D}{dt} = \beta(m_D - p_D)$$

$$\frac{dm_E}{dt} = \frac{\alpha_C p_C^n}{(1 + p_A^n)(1 + p_B^n)(1 + p_C^n)(1 + p_D^n)} - m_E \qquad\qquad \frac{dp_E}{dt} = \beta(m_E - p_E)$$

The classifier decision will be defined as follows: if $p_E > p_D$ we say the output is OFF, while if $p_E < p_D$ then the output is ON. Following the setting of [10,11], where inputs are constant in time, initial conditions in the threshold unit are equal and their transcription strength α_i from gene $i = A, B, C$ also contribute equally, the relation between values of p_D and p_E is determined by the sign of the linear expression $p_A + p_B - p_C$. This comes from the fact that the basal expression of gene C is constant so that the gene expression E is greater than D. Nevertheless, if the inputs A and B are presented such that repress gene E in spite of the influence from C, the system will switch its state and the gene D will be greater than E.

Thus, under these conditions, a reference is defined according to the expression:

$$p_A + p_B < p_C \implies p_D < p_E$$

$$p_A + p_B > p_C \implies p_D > p_E \tag{2}$$

$$p_A + p_B = p_C \implies p_E = p_D$$

As the inputs from A and B are presented, their signals are superimposed and compared against a threshold, from where we get two possible outcomes (ON or OFF). In this sense (2) is used as reference: if this relation is satisfied then the answer is denoted as *correct*. Thus, this system can function as a binary classifier. In the next section we will observe that under the same conditions on the inputs and threshold, if the system (2.1) is in equilibrium, then the same rule of classification can be resolved when there is not cooperativity binding and transcription strengths are equal. Importantly, observe from (2.2) that in equilibrium the inputs p_A and p_B coincide with the basal activity a_A and a_B of their respective genes, while the threshold given by p_C will reach a_C.

3. Dynamical analysis

3.1. Equilibrium state

Assuming the set of equations (1) in equilibrium we can obtain a 2-dimensional algebraic system of equations to solve for p_E and p_D in order to obtain their protein concentration in steady state. This follows from the fact that A, B and C are uncoupled from the system, so that the equilibrium points would depend on the contributions from gene E and D. In turn, the problem can be analyzed in this reduced system.

$$\frac{\alpha_C a_C{}^n}{(1 + a_A{}^n)(1 + a_B{}^n)(1 + a_C{}^n)(1 + p_D{}^n)} = p_E \tag{1}$$
$$\frac{\alpha_A a_A{}^n + \alpha_B a_B{}^n}{(1 + a_A{}^n)(1 + a_B{}^n)(1 + a_C{}^n)(1 + p_E{}^n)} = p_D$$

Observe that according to (1), the protein inputs from genes A and B in steady state are a_A and a_B, respectively while for gene C is a_C. Observe that the equilibrium protein concentrations for genes D and E can be obtained for each n by substitution of the first equation for p_E into the the second one. Once the system reaches the equilibrium, protein levels of D and E are compared in order to define an output. In this paper we use (3.1) to construct the associated nullclines in the 2-dimensional system described by p_D and p_E. The equilibrium points were obtained numerically and their stability was assessed by determining the direction of the vector field in all regions, specifying a control parameter of interest.

Observe that since genes A, B and C are uncoupled from the system, the analysis of (2.1) can be reduced in complexity by just considering equilibrium of genes D and E. In turn, the dynamical properties on this model will be studied in the space (p_D, p_E).

3.2. Rule of classification

From the set of algebraic equations (1) it is possible to derive a general rule for binary classification for the present model. Considering the first equation of the system (1) we obtain,

$$\frac{1}{(1 + a_A{}^n)(1 + a_B{}^n)} = \frac{p_E(1 + a_C{}^n)(1 + p_D{}^n)}{\alpha_C a_C^n} \tag{2}$$

by substituting (2) in the second equation of (1) and after some arrangements is possible to arrive to the expression:

$$\bar{\alpha_A} a_A{}^n + \bar{\alpha_B} a_B{}^n = \frac{p_D(1 + p_E{}^n)}{p_E(1 + p_D{}^n)} a_C{}^n \tag{3}$$

where $\bar{\alpha_i} = \frac{\alpha_i}{\alpha_C}$ for $i = A, B$.

Parameters a_A and a_B represent protein inputs from genes A and B, respectively. In addition, each one is weighted by a coefficient $\bar{\alpha_i}$ and the threshold is defined by $a_C{}^n$. From equation (1) we have that by specifying an input with fixed weights, we can solve for p_E and p_D in order to get their equilibrium protein concentrations $p_E{}^*$ and $p_D{}^*$ and thus the output of classification.

Observe that the dynamical behaviour of the system will show bistability due the toggle switch $E - D$ [15]. This means that the joint action of A and B switches the state to ON or OFF for $n > 1$. This switch is due to an unbalanced promoter strength. When such a balance is attained we have bistability and the condition $p_E{}^* = p_D{}^*$ is satisfied. These states lie in a line that separates both the ON and OFF outputs setting the threshold for the system at different values of cooperativity. Therefore, from (3.3) it follows that,

$$\bar{\alpha_A} a_A{}^n + \bar{\alpha_B} a_B{}^n = a_C{}^n \tag{4}$$

Once the system is unbalanced the system will have a unique steady state for proteins D and E. Due to the permanent basal activity of gene C if this threshold is not surpassed by action of the inputs, then the output will remain as OFF. The latter observations lead to a more general rule of classification for the proposed model.

$$\bar{\alpha_A} a_A{}^n + \bar{\alpha_B} a_B{}^n < a_C{}^n \Leftrightarrow p_D{}^* < p_E{}^* \ (OFF\ state) \tag{5}$$
$$\bar{\alpha_A} a_A{}^n + \bar{\alpha_B} a_B{}^n > a_C{}^n \Leftrightarrow p_D{}^* > p_E{}^* \ (ON\ state)$$

In particular, for the linear case using $n = 1$ in (3.3) that $\frac{p_D{}^*(1 + p_E{}^*)}{p_E{}^*(1 + p_E)} > 1$ which implies that $p_D{}^* > p_E{}^*$, while if $p_E{}^* > p_D{}^*$ we have $\frac{p_D{}^*(1 + p_E{}^*)}{p_E{}^*(1 + p_D{}^*)} < 1$. Thus, with our model we can resolve the rule of classification (2.2) including constants related with the biochemical interactions, which is a novel result.

Parameter $a_C{}^n$ defines such threshold, in which we take $a_C = 1$, by simplicity. If we additionally assume that the transcription coefficients α_i are all equal for $i = A, B, C$, then we arrive to the equation $a_A{}^n + a_B{}^n = 1$, which as observed for the linear case, it defines a line of separation of between the ON and OFF states. In figure 2 we show the curves for this function for different levels of cooperativity. According to (5), the interior of each curve given by $a_A{}^n + a_B{}^n < 1$ defines the OFF state and $a_A{}^n + a_B{}^n > 1$ the ON state. Thus, we observe that the cooperativity binding plays an important role for the binary classification for this model, and the combination of pairs of inputs (a_A, a_B) may lie in different states (either ON or OFF) as we vary this parameter. This is in contrast with the results provided by [10]. More precisely, observe that by varying constants $\bar{\alpha_A}$, $\bar{\alpha_B}$ and a_C it is possible to obtain different shapes defined by the curve of separation from equation (4). Note that this could enable a construction of distributed genetic

classifiers as suggested in [16]. An important goal in synthetic biology is the design and construction of genetic networks with a desired ability. In the present work we introduce a model for a synthetic genetic classifier in (1) using a standard approach. We show that classification can be manipulated given some control parameters related with transcriptional mechanisms.
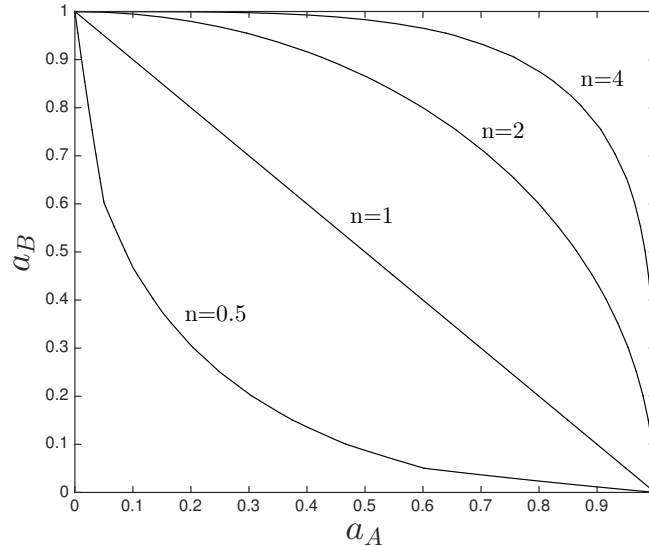

FIGURE 2. The separation curve for each n divides states ON and OFF when the inputs A and B are in steady state. The exterior of each one will determine the ON state and the interior the OFF state. The curves depend on the weights with which inputs inhibit gene D and activate gene E, and on the threshold of gene C. Parameters $\bar{\alpha_A} = \bar{\alpha_B} = 1$ and $a_C = 1$ describe the weight of the inputs and threshold, respectively.

3.3. Influence of the inputs and weights in the equilibrium state

Accuracy of classification for a given input is obtained by comparing the correct answer with the obtained output. According to [10, 11] it is discussed that such accuracy is decreased by the influence of bistability due to the toggle switch E-D, so that switching among steady states is observed. This means that bistability is expected for $n > 1$, while in the linear case (noncooperative binding) we have monostable dynamics.

The specific case $n = 2$ for equation (3) is analyzed. Here the transcription rates a_i and basal activity α_i for $i = A, B$ are considered as control parameters. The effect of a_C and α_C in the classification can be observed from (5): parameter a_C defines the threshold of classification and α_C scales the weights α_i of inputs from gene $i = A, B$. For simplicity, we shall assume them fixed $a_C = \alpha_C = 1$. In the following we investigate the influence of such parameters in the equilibrium states of protein D and E $(p_D{}^*, p_E{}^*)$, solutions of (3.1).

Firstly, as we vary the input value, e.g., a_B, for fixed $\alpha_i = 20$, for all i (in turn $\bar{\alpha}_i = 1$), there are three different states $p_D{}^*$, two stable $p_D{}^* = 0.24, 4.21$ and one unstable $p_D{}^* = 1.44$, which remain constant for all α_B. The same result holds for a_A due to the symmetry in the system. By substitution of each equilibrium concentration $p_D{}^*$ in the first equation of (3.1) it is observed that protein concentration in E will hold the same dynamical behaviour.

By using (1) it is possible to obtain the nullclines showing the set of equilibrium states $(p_E{}^*, p_D{}^*)$ to describe the dynamics of the entire system. In particular, in order to have an insight into the behaviour

of the separation curve we choose a fixed input from this one $(1/\sqrt{2}, 1/\sqrt{2})$. Nullclines $\frac{dm_D}{dt} = \frac{dm_E}{dt} = 0$ intersect at three points: one unstable and two stable steady states. Then for this set of parameters bistability is observed in figure 3 (a), due to the cooperativity and balanced rate of synthesis. The balance may be lost as we vary the transcription rate for gene A and B leading to obtain a single steady state. This can be seen from figure 3 (b).

The bifurcation plot using $\alpha_A = \alpha_B = \alpha$ as a control parameter with $a_A = a_B = 1/\sqrt{2}$ and $\alpha_C = 20$ is showed in figure 4. A bistability can be observed as we vary the value of transcription rates α. For the parameters considered we reach the bistability region within the interval delimited by $\alpha^* = 15.46$ and 31.50. Thus, errors of classification are expected due to switching between stable states. Furthermore, due to the toggle switch E-D, according to [15] higher order cooperativity it is possible to obtain bistability for lower α while broadening the bistable region.
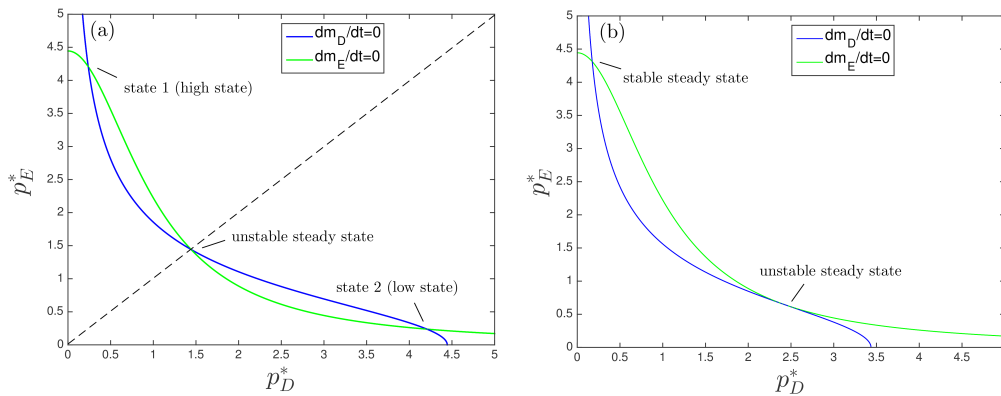

FIGURE 3. (a) A bistable nonlinear classifier. Nullclines defining the solutions $\frac{dm_D}{dt} = \frac{dm_E}{dt} = 0$ for $\alpha_A = \alpha_B = 20$; (b) A nonlinear classifier at the bifurcation point $\alpha_A = \alpha_B = 15.46$. Other parameters are fixed inputs $(1/\sqrt{2}, 1/\sqrt{2})$, $n = 2$, $\beta = 5$ and $\alpha_C = 20$ and $a_C = 1$.

4. Effect of intrinsic noise in the classification

4.1. Methods

Our findings so far show that the considered model for the binary classifier is able to discriminate two external stimuli in terms of a rule given in (5). This is true in the deterministic case, which has been already discussed. On the other hand, it is of interest to assess the ability of classification under the effects of intrinsic fluctuations due to low copy number of biochemical species. For this matter, the setting of p_A and p_B, and threshold p_C was considered in steady state (static classification) and initial concentrations for gene D and E are assumed equal by simplicity. Additionally, we set coefficients $\alpha_i = 1$ for $i = A, B, C$. Recall that in steady state p_i coincides with a_i for $i = A, B$.

In this paper, we introduce intrinsic noise using the Gillespie's algorithm and Chemical Langevin Equation (CLE) [17, 18] by employing the Hill functions obtained in model (2.1). First, the Gillespie algorithm can be performed assuming the set of reactions of table 1. This table indicates that from gene
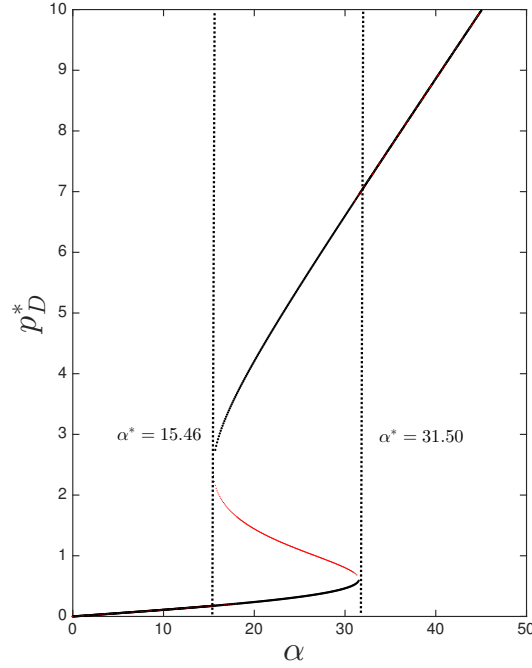
FIGURE 4. Bifurcation plot for binary classification with $n = 2$. The control parameter is given by $\alpha = \alpha_A = \alpha_B$. Other parameters are fixed inputs $(1/\sqrt{2}, 1/\sqrt{2})$, $\beta = 5$, $\alpha_C = 20$ and $a_C = 1$.

$i = A, B, C, D, E$ the process of transcription leads to production of mRNA m_i followed by translation to protein p_i. Degradation leads to a product ϕ which is not taken into account. The indicated propensities were obtained from the corresponding model (2.1) in the standard way.

Therefore, CLE can be computed from the propensities of Table 1.

$$\frac{dm_A}{dt} = (a_1 - a_6) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_1}\xi_1(t) - \sqrt{a_6}\xi_6(t)] \qquad \frac{dp_A}{dt} = (a_{11} - a_{16}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_{11}}\xi_{11}(t) - \sqrt{a_{16}}\xi_{16}(t)]$$

$$\frac{dm_B}{dt} = (a_2 - a_7) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_2}\xi_2(t) - \sqrt{a_7}\xi_7(t)] \qquad \frac{dp_B}{dt} = (a_{12} - a_{17}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_{12}}\xi_{12}(t) - \sqrt{a_{17}}\xi_{17}(t)]$$

$$\frac{dm_C}{dt} = (a_3 - a_8) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_3}\xi_3(t) - \sqrt{a_8}\xi_8(t)] \qquad \frac{dp_C}{dt} = (a_{13} - a_{18}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_{13}}\xi_{13}(t) - \sqrt{a_{18}}\xi_{18}(t)]$$

$$\frac{dm_D}{dt} = (a_4 - a_9) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_4}\xi_4(t) - \sqrt{a_9}\xi_9(t)] \qquad \frac{dp_D}{dt} = (a_{14} - a_{19}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_{14}}\xi_{14}(t) - \sqrt{a_{19}}\xi_{19}(t)]$$

$$\frac{dm_E}{dt} = (a_5 - a_{10}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_5}\xi_5(t) - \sqrt{a_{10}}\xi_{10}(t)] \qquad \frac{dp_E}{dt} = (a_{15} - a_{20}) + \frac{1}{\sqrt{\Omega}}[\sqrt{a_{15}}\xi_{15}(t) - \sqrt{a_{20}}\xi_{20}(t)]$$

where $\xi_{i=1,\dots,20}(t)$ are uncorrelated Gaussian white noises $< \xi_i(t)\xi_j(t') > = \delta_{ij}\delta(t - t')$ where coefficients $\xi_{i=1,\dots,20}(t)$ provide the intrinsic noise scaled by $1/\sqrt{\Omega}$, and Ω denotes the size of the system.

For the small size system (denoted by Ω) Gillespie's algorithm can be used as a standard method whereas for a large system size CLE is computationally more efficient and provides a good approximation. Classification of the inputs is studied for the case of Hill coefficient $n = 1$ and $n = 2$ obtained from a set of 10^3 data points per each input protein in the duple (a_A, a_B). Each protein input is uniformly distributed in $[0, 1]$ and in turn time series for p_E and p_D are obtained. Thus, linear classification $(n = 1)$ is studied under the influence of intrinsic noise by using CLE for size system $\Omega = 10^3$ and Gillespie's algorithm for $\Omega = 500$. In addition, stochastic integration was obtained by Euler-Maruyama method.

Reaction	Propensity
$[A] \to m_A$	$a_1 = a_A$
$[B] \to m_B$	$a_2 = a_B$
$[C] \to m_C$	$a_3 = a_C$
$[D] \to m_D$	$a_4 = \dfrac{(\alpha_A p_A^n + \alpha_B p_B^n)}{(1 + p_A{}^n)(1 + p_B^n)(1 + p_C^n)(1 + p_E^n)}$
$[E] \to m_E$	$a_5 = \dfrac{\alpha_C p_C^n}{(1 + p_A^n)(1 + p_B^n)(1 + p_C^n)(1 + p_D^n)}$
$m_A \to \phi$	$a_6 = m_A$
$m_B \to \phi$	$a_7 = m_B$
$m_C \to \phi$	$a_8 = m_C$
$m_D \to \phi$	$a_9 = m_D$
$m_E \to \phi$	$a_{10} = m_E$
$[m_A] \to p_A$	$a_{11} = \beta m_A$
$[m_B] \to p_B$	$a_{12} = \beta m_B$
$[m_C] \to p_C$	$a_{13} = \beta m_C$
$[m_D] \to p_D$	$a_{14} = \beta m_D$
$[m_E] \to p_E$	$a_{15} = \beta m_E$
$p_A \to \phi$	$a_{16} = \beta p_A$
$p_B \to \phi$	$a_{17} = \beta p_B$
$p_C \to \phi$	$a_{18} = \beta p_C$
$p_D \to \phi$	$a_{19} = \beta p_D$
$p_E \to \phi$	$a_{20} = \beta p_E$

TABLE 1. Stochastic version of the the synthetic genetic classifier model

4.2. Results and discussion

Stochastic simulations for $n = 1$ are shown in figure 5 (fig. 5 (a) corresponds to $\Omega = 10^3$ and fig. 5 (b) to $\Omega = 500$). It is observed that the binary classifier is able to perform linear separation on the inputs for different levels of noise. Here, the ON state lies in the blue area while the OFF state in the red area. Moreover, this separation is comparable with results in the deterministic case (figure 2). Nevertheless, in figure 5 it is observed that the influence of intrinsic noise blurs the vicinity of the line of classification for both plots, which increases for a larger level of noise. This trend in the line of classification is similar to the results found for the genetic perceptron modeled in [10].

This study can be extended by including different levels of cooperativity. By simplicity, we investigate positive cooperativity with $n = 2$, which was already discussed in section 3 for the deterministic case. The classification plot is shown in figure 6, which is obtained by binary discrimination of 10^3 data points using CLE simulation with $\Omega = 10^3$. As observed, the system can perform nonlinear classification. Using the aforementioned set of parameters and (4) the line of classification for the deterministic case is given by $a_A{}^2 + a_B{}^2 = 1$. The obtained results show that this trend is preserved and as in the case of $n = 1$ the vicinity in the line of classification is blurred. As a consequence, the effect of intrinsic noise leads to reducing the accuracy of classification as previously noticed for $n = 1$ and $\Omega = 500$. Nevertheless, the nature of noise is fundamentally different for both cases. In fact, the influence of intrinsic noise for this genetic network may be analyzed for two main cases: $n \leq 1$ (monostable dynamics) and $n > 1$ (bistable dynamics).

In the former case, $n \leq 1$, according to the toggle switch E-D, there is a unique stable state. That is, the system is monostable and in this way there is not switching between equilibria. For instance, by taking $n = 1$, $\alpha_i = 20$ for $i = A, B, C$ and $\Omega = 50$ inputs lie in the line of classification (inputs $a_A = a_B = 0.5$ are chosen for simulations). Errors of classification may be expected for points in the vicinity of the line of classification (figure 7 (a)-(c)). Results in the deterministic case show that both protein concentration p_D and p_E are equal in the line of classification. Then, the difference at an arbitrary time is null. However, by adding intrinsic noise it is clear that the time series for concentration of p_D and p_E will not be equal. Instead, discrepancies due to fluctuations are observed, and those are increased as Ω is reduced. In turn, the difference between both times series will be a random variable for each time t. Thus, for inputs lying out of the line of separation, signals p_D and p_E may overlap at random times because of fluctuations for given levels of noise. As a consequence the vicinity of the line of classification is expected to have
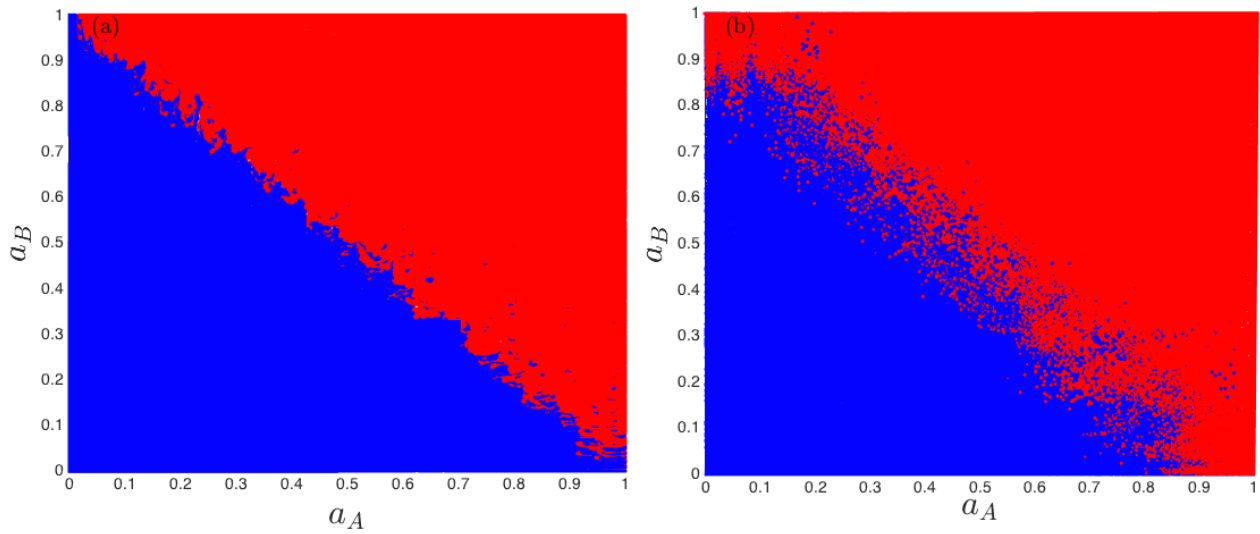
FIGURE 5. Linear classification performed by binary classifier ($n = 1$) under the effects of intrinsic noise: (a) $\Omega = 10^3$ simulated by Chemical Langevin equation; (b) $\Omega = 500$, by Gillespie's algorithm.
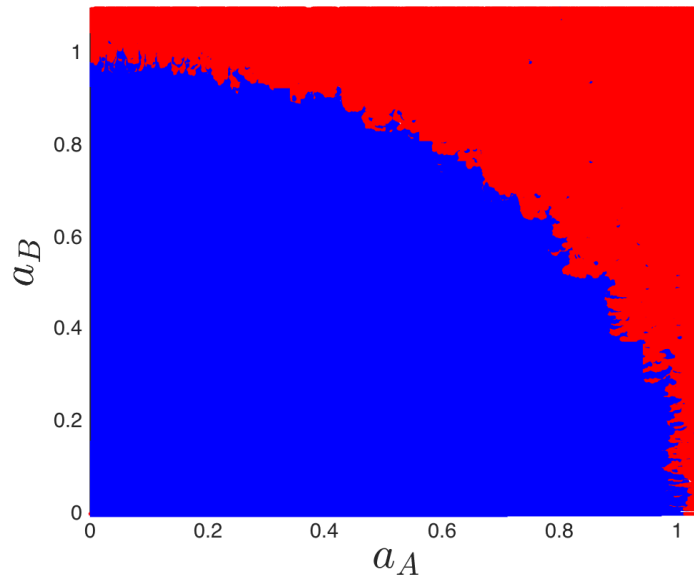
FIGURE 6. Classification performed by binary classifier ($n = 2$) under the effects of intrinsic noise $\Omega = 10^3$ simulated by Chemical Langevin equation.

this blurred trend. Namely, the accuracy for CLE simulations is expected to depend on the factor $1/\sqrt{\Omega}$, which gives the level of noise in terms of the system size.

Moving towards the case of positive cooperativity, it is possible to have bistability as we vary the transcriptional strength α_i from genes $i = A, B$. In figure 6 we choose $\alpha_i = 20$ for $i = A, B, C$ and cooperativity coefficient $n = 2$. According to (3.4) we expect that the curve of separation is represented by the equation $a_A{}^2 + a_B{}^2 = 1$, dividing the states ON and OFF. In fact, this curve is comparable with our predictions as the system is able to perform nonlinear classification as described in (3.5). However, we observe a blurred trend in the boundary that separates both states. This is the case where $p_E{}^* = p_D{}^*$. In principle this is attributed to bistability, which introduces error in the process of classification since there is a continuous switching between steady states as observed in 7 (b). In this case we take input concentrations lying in the curve of separation, in particular we use $a_A = a_B = 1/\sqrt{2}$ and transcriptional strengths balanced.

In figure 7 (c) and (d) we show nullclines for $n = 1$ and 2 to describe the monostable and bistable dynamics of the system. When there is non-cooperativity binding is expected to have a single steady state, while for the case $n = 2$ we have that bistability is achieved so that there are three different steady states (two stable states and one unstable state). As a consequence, as we introduce noise it is observed a switching between the high and low stable equilibrium states which blurs the curve of separation and introduces error in the classification process. The underlying mechanism of the stochastic switch relies in the fact that fluctuations become considerable enough such that a specific microstate around the stable equilibrium of the system crosses the separatrix to another microstate around the second stable equilibrium [19].
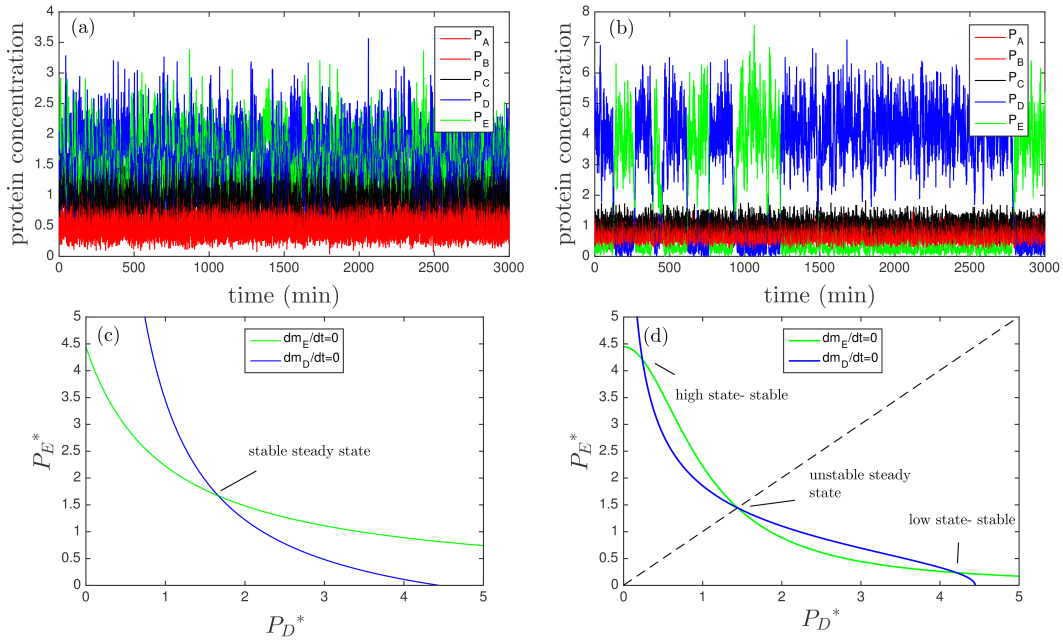

FIGURE 7. Protein time series of binary classifier using Gillespie's algorithm (a) $n = 1$ with inputs $a_A = a_B = 1/2$; (b) $n = 2$ with inputs $a_A = a_B = 1/\sqrt{2}$; (c) A monostable classifier. Nullclines defining the solutions $\frac{dm_D}{dt} = \frac{dm_E}{dt} = 0$ for $\alpha_A = \alpha_B = 20$ and $n = 1$ with same inputs as (a); (d) A nonlinear classifier at the bifurcation point $\alpha_A = \alpha_B = 15.46$ and $n = 2$ with same inputs as (b). For the nullclines other parameters are $\beta = 5$, $\alpha_C = 20$ and $a_C = 1$.

Bistability introduces another way to provide error in the binary classifier. In particular, as we introduce inputs p_A and p_B, if their combined action lie in the vicinity of the line of separation of ON-OFF states (figure 2), it may be possible to get outputs that evolve alternating between correct and incorrect answers

as we introduce intrinsic noise. The rate of switching between such states increases in terms of the level of noise. As a consequence, the blur trend around the line of separation increases as shown in figure 5 and 6. This means that the quality of classification is diminished.

Besides this apparent disadvantage, it would be of interest to discuss the usefulness that this switching provides in the classification process. In biological systems phenotypic switching in cellular decision-making can be advantageous. In [1] some examples of interest are provided including resource optimization in multicellular organisms [20] during starvation and persistence under fluctuating environments [21]. Our system is suitable to mimic processes that involve cell fate decisions, since it counts with a classifier depicted as a feedback loop which evaluate inputs that act as external sources. On this context, this motivates the using of (4) and (5) to model decision-making associated with switching between two distinct cell states under proper conditions. Thus, if the joint contribution of inputs (4) is close to a defined threshold, when noise is present switching will occur as the interactions are sufficiently symmetric with respect to the toggle switch E-D. This may provide an insight into the understanding and altering of noise in a feasible way in order to control cell fate decisions, which is of current interest.

Concrete applications of this idea are encountered in bet-hedging decisions [22, 23], in which stochastic fluctuations for adequate levels of noise allows switching into a evolutionary stable environment. One remarkable example is bacteria persistence such as the case of *Bacillus subtilis* [5]. As nutrients decrease, B. subtilis starts a process of sporulation, which is delayed by searching other feasible options. In the case cells cannot adapt to their environment such as poor nutrient conditions, there is promotion of random mutations. On of these cell fate decision is the transition to competence [2], which means that cells obtain resources from the extracellular DNA to use it either as food or evolvability [24]. Some clonal bacterial population act as competent while others present sporulation, and in this case, an appropiate level of noise leads to aid survival, however, reduction of noise is followed by a reduction of competent cells [2, 25] which mean a reduction in the chances of survival.

The optimality of decision making of our model under the influence of noise will depend on the proper control of switching times and duration. A random switch to an incorrect state at the wrong time could have negative consequences. For instance, during the increase of cell population during eukaryotic development there are serious danger of error amplification. The assignation of cell fates during this process must rely in control mechanisms that filter noise in order to have a coordinated and stable gene expression [26]. In our network, these mechanisms are related with the model parameters for a fixed level of noise as observed in section 3.3; and the level of noise is proportional with the rate of switching. Nevertheless, this topic is out of scope this paper. Further research for the rate of random phenotypic switching and duration would be useful in order to achieve optimal synthetic systems for cell fate decisions.

Our findings give the possibility to consider a more general case than the original model [10]. Namely, it has the virtue of modifying the area in which one decision lies (ON/OFF states) in terms of the transcription rates of the genes involved as formulated in (1). Binary classification using this approach is very recent to our understanding and may result advantageous for the construction of synthetic networks. Furthermore, cell decision making has already been discussed in a biological context. However, here we propose a model capable to discriminate an output with a diversity of parameters to set a rule of classification; and in which intrinsic noise plays a functional role for bistability at the level of gene regulation. As a prospect, it would be of interest to analyze how the switching takes place in a particular state in terms of the level of noise and given parameters.

5. Conclusions

In this paper we emphasized in the influence of intrinsic noise in a genetic perceptron, a synthetic genetic network which is able to perform classification of external stimuli. We derived a dynamical model for this system using Hill functions to describe the switch-like gene responses and the intracellular chemical reactions. We analyzed the ability of this system to perform classification of stimuli in steady state. In particular, we formulated a rule of classification which is obtained in terms of biological parameters of the threshold gene and inputs. It is found that classification can be performed when the system is either monostable or bistable. We studied qualitatively the effects of intrinsic noise for the classification accuracy. We find that high levels of noise may lead to an important detriment of the classification accuracy. We discuss potential applications regarding the classification under the effect of intrinsic noise and bistability.

References

[1] G. Balázsi, A. van Oudenaarden, J.J. Collins. *Cellular decision making and biological noise: From microbes to mammals.* Cell, 144 (2011), 910–925.

[2] G.M. Süel, J. Garcia-Ojalvo, L.M. Liberman, M.B. Elowitz. *An excitable gene regulatory circuit induces transient cellular differentiation.* Nature, 440 (2006), 545–550.

[3] S.L. Spencer, S. Gaudet, J.M. Burke, P.K. Sorger. *Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis.* Nature, 459 (2009), 428–432.

[4] M. Cohen, M. Georgiou, J.M. Burke, N.L. Stevenson, M. Miodownik, B. Baum. *Dynamic filopodia transmit intermittent delta-notch signaling to drive pattern refinement during lateral inhibition.* Dev. Cell, 19 (2010), 78–89.

[5] N.Q. Balaban, J. Merrin, R. Chait, L. Kowalik, S. Leibler. *Bacterial persistence as a phenotypic switch.* Science, 305 (2004), 1622–1625.

[6] M. Thattai, A. van Oudenaarden. *Intrinsic noise in gene regulatory networks.* Proc. Natl. Acad. Sci. USA, 98 (2001), 8614–8619.

[7] M. Kærn, T.C. Elston, W.J. Blake, J.J. Collins. *Stochasticity in gene expression: From theories to phenotypes.* Nat. Rev. Genet., 6 (2005), 451–464.

[8] J.M. Raser, E.K. O'Shea. *Noise in gene expression: origins, consequences, and control.* Science, 309 (2005), 2010–2013.

[9] V. Ambravaneswaran, I.Y. Wong, A.J. Aranyosi, M. Toner, D. Irimia. *Directional decisions during neutrophil chemotaxis inside bifurcating channels.* Integr. Biol., 2 (2010), 639–647.

[10] R. Bates, O. Blyuss, A. Alsaedi. *Effect of noise in intelligent cellular decision making.* PLoS One, 10 (2015), e0125079.

[11] S. Filicheva, A. Zaikin, O. Kanakov. *Dynamical decision making in a genetic perceptron.* Physica D: Nonlin. Phenom., 318-319 (2016), 112–115.

[12] V. Samborska, S. Gordleeva, E. Ullner, A. Lebedeva, V. Kazantsev, M. Ivanchenko, A. Zaikin. *Mammalian brain as a network of networks.* Opera Med. Physiol., (2016), 23–38.

[13] N. Suzuki, C. Furusawa, K. Kaneko. *Oscillatory protein expression dynamics endows stem cells with robust differentiation potential.* PLoS One, 6 (2011), e27232.

[14] M. Santillán. *On the use of the hill functions in mathematical models of gene regulatory networks.* Math. Model. Nat. Phenom., 3 (2008), 85–97.

[15] T.S. Gardner, C.R. Cantor, J.J. Collins. *Construction of a genetic toggle switch in Escherichia coli.* Nature, 403 (2000), 339–342.

[16] O. Kanakov, R. Kotelnikov, A. Alsaedi, L. Tsimring, R. Huerta, A. Zaikin, M. Ivanchenko. *Multi-input distributed classifiers for synthetic genetic circuits.* PLOS ONE, 10 (5) (2015), e0125144.

[17] D.T. Gillespie. *The chemical Langevin equation.* J. Chem. Phys., 113 (2000), 297–306.

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

[19] S. Waldherr, J. Wu, F. Allgöwer. *Bridging time scales in cellular decision making with a stochastic bistable switch.* BMC Syst. Biol., 4 (2010), 108.

[20] T. Çağatay, M. Turcotte, M.B. Elowitz, J. Garcia-Ojalvo, G.M. Süel. *Architecture-dependent noise discriminates functionally analogous differentiation circuits.* Cell, 139 (2009), 512–522.

[21] M. Acar, A. Becskei, A. van Oudenaarden. *Enhancement of cellular memory by reducing stochastic transitions.* Nature, 435 (2005), 228–232.

[22] E. Kussell, S. Leibler. *Phenotypic diversity, population growth, and information in fluctuating environments.* Science, 309 (2005), 2075–2078.

[23] T.J. Perkins, P.S. Swain. *Strategies for cellular decision-making.* Mol. Syst. Biol., 5 (2009), 1–15.

[24] R.S. Galhardo, P.J. Hastings, S.M. Rosenberg. *Mutation as a stress response and the regulation of evolvability.* Crit. Rev. Biochem. Mol. Biol., 42 (2007), 399–435.

[25] H. Maamar, A. Raj, D. Dubnau. *Noise in gene expression determines cell fate in bacillus subtilis.* Science, 317 (2007), 526–529.

[26] A. Arias, P. Hayward. *Filtering transcriptional noise during development: concepts and mechanisms.* Nat. Rev. Genet., 7 (2006), 34–44.