Synthetic bio-computation design using supervised gene regulatory networks

Luis F. Seoane1,2 and Ricard V. Sole1,2,3
1ICREA-Complex Systems Lab, Universitat Pompeu Fabra, Dr Aiguader 88, 08003 Barcelona, Spain
2Institut de Biologia Evolutiva, UPF-CSIC, Psg Barceloneta 37, 08003 Barcelona, Spain
3Santa Fe Institute, 1399 Hyde Park Road, Santa Fe NM 87501, USA

The potential of synthetic biology techniques for designing complex cellular circuits able to solve complicated computations opens a whole domain of exploration, beyond experiments and theory. Such cellular circuits could be used to carry out hard tasks involving decision-making, storage of information, or signal processing. Since Gene Regulatory Networks (GRNs) are the best known technical approach to synthetic designs, it would be desirable to know in advance the potential of such circuits in performing tasks and how classical approximations dealing with neural networks can be translated into GRNs. In this paper such a potential is analyzed. Here we show that feed-forward GRNs are capable of performing classic machine intelligence tasks. Therefore, two important milestones in the success of Artificial Neural Networks are reached for models of GRNs based on Hill equations, namely the back-propagation algorithm and the proof that GRNs can approximate arbitrary positive functions. Potential extensions and implications for synthetic designs are outlined.

Keywords: Biological computation, neural networks, synthetic biology, gene regulation

I. INTRODUCTION

Cells are entangled living machines capable of very complex computational tasks. They rival parallel computers and are in charge of the fine tuned responses that allow them, along with tissues and organs, to properly adapt to external and internal challenges (Bray 1990, Bray 1995). The idea that complex patterns of cellular behavior can be an emergent property of gene-gene interactions was early proposed by Stuart Kauffman, who used a Boolean approximation to gene regulation as a minimal model of the true (and complex) molecular events (Kauffman 1993). This view was originated shortly after the classical work by Warren McCulloch and Walter Pitts, which showed that any particular computational task (as defined by a logic gate) can be mapped into a threshold-like neural network (McCulloch and Pitts 1943). It is interesting to notice that, since those early years, both neural and genetic networks have received an always increasing attention both at the level of the details of their interacting constituents as well as in terms of theoretical models. In both cases, it is often possible to consider that each element (either a formal neuron or a simplified gene, which we shall call indistinctly unit throughout the text) responds to external stimuli in nonlinear ways. The associated response functions that characterize both types of units are commonly stepwise, ideally Boolean-like.

The theory of neural networks rapidly advanced, primarily thanks to the development of computers and simulation techniques. In parallel, neuroscience actively studied the behavior of nerve cells with outstanding precision. At the end of the 20th century, neuron-based models were highly accurate and essentially well established (Dayan and Abbott 2005). Moreover, several standard approximations emerged towards the distributed solution of a plethora of computation problems (Bishop 2006): leaving aside elemental classification and interpolation tasks based on sample data; Artificial Neural Networks (ANNs) were successfully applied on signal processing, pattern recognition, complex inference, nonlinear control, etc. By contrast, gene networks received less consideration since molecular biology started to dominate the scene from the 1950s. More and more attention was being paid to how single genes worked and a largely reductionistic agenda was developed. With the discovery of gene regulation by Jacob and Monod (Jacob and Monod 1961), the picture started to (very) slowly move towards a circuit-based functional view of cellular control. The gene network view has been ever since gradually adopted by most biologists, who became aware of the dominant role played by gene-gene interactions.

Gene Regulatory Networks (GRNs) are a holistic conceptualization of large assemblies of interacting genes and their regulatory interplay (Kitano 2001, Alon 2006). They are the result of a non-designed process of evolutionary tinkering (Rodriguez-Caso et al. 2005) and thus they display some non-standard patterns of network organization. Specific mathematical modeling can be made thanks to the tools already developed for single gene and other molecular dynamics characterization: we will be working with the quite standard and successful Hill differential equation (Hill 1910). Reconstruction of small motifs from real genetic networks (Alon 2007), and also of each time larger interwoven collections of genes (Saez-Rodriguez et al. 2009) has become possible using experimental data. Small feed-forward motifs like those represented in figure 1 are overly abundant in large GRNs (Macia et al. 2009a, Widder et al. 2012), and they re-
semble us enough of the synthetic networks that we will be designing. The connection that we intend to make between GRNs and machine learning is already hinted at by AI methods for gene-webs reconstruction (Sirbu et al. 2011 and references therein), where ANNs reveal themselves as a very appropriate model of GRNs.

The understanding of how GRNs work helps us build a much better picture of how computations occur in living cells (Bray 1990; Bray 1995; Nurse 2008; Brenner 2012) and invites us to think of GRNs from an engineering standpoint. In recent years ambitious calls and claims have been made that pursue the implementation of actual biological computing devices (Amos 2004; Amos 2008; Benenson 2012). These would use interconnected genes, assemblies of cells, and alike to reckon and execute controlled responses to external conditions. One final goal would be to elaborate computing resources that could be easily integrated into living organisms, although up to date most of these contributions have been developed in vitro. Even in an artificial environment free from the unpredictability of complex organisms, the components that nature provides us with present a series of important drawbacks: our theoretical understanding of cellular processes is largely incomplete; we usually handle leaky, diffusing systems where a precise spatial architecture becomes difficult, if not impossible; chemical reactions are essentially stochastic; etc. Thus, seemingly simple designs have required very rigorous experimental controls (Rothemund et al. 2004; Bratsun et al. 2005; Tabor et al. 2009; Mondragón et al. 2011). Major progresses might require non-standard approximations (Regot et al. 2010; Macía et al. 2012) that liberate us from any of the existing constrains, or to novel technical advances, such as multiples genome engineering (Wang et al. 2009). The exhaustive experimental (Hoffman-Sommer et al. 2012) or theoretical (Hasty et al. 2001) characterization of specific existing circuits (both in vitro and in silico) is also necessary.

In this paper we tackle theoretical issues regarding the most basic designable computational capabilities of GRNs. We restrict ourselves to feed-forward networks, which lack feedback loops and thus will not exhibit dynamical behaviors other than steady state attractors. We present an error back-propagation algorithm to design actual GRNs that would solve specific problems. Experimentally hard wiring a set of parameters into a synthetic biological system seems a little ahead of schedule, but the development of genetic engineering techniques that allow a combinatorial design of complex circuits is becoming a reality (Mattussi and Floreano 2007; Wang et al. 2009) and we shall soon have the potential of creating any requested synthetic GRN. Similarly, synthetic customizable signaling networks are becoming a reality (Bashor et al. 2010; Lim 2010). Although combinatorial design with biological components is still in its infancy, the potential for complex computational synthetic networks is growing fast (Solé and Macía 2013). The questions we pose are: How do these gene-based circuits need to be designed and to what extent can they perform computational tasks similar to those performed by ANNs? Are these tasks easy to implement by GRN? What are the design rules required to obtain the optimal designs?

We close this introduction outlining that GRNs are just one of the many biological structures that seem an appropriate substrate to implement AI means. We can think of others, such as transduction networks, that can also be modeled up to a great detail using Hill equations. Hill functions will be our main tool in the current paper, thus our developments should apply not only to GRNs, but to many more systems.

The article is structured as follows: In section II it is shown in a constructive way how a feed-forward network of Hill equations can approximate any arbitrary continuous positive function. In section III the back-propagation rules are explicitly derived for our mathematical model of GRNs and in section IV we show how our idea was applied (of course, in silico) to one fitting problem and three classification tasks. Discussion and future lines of work follow in section V.

II. FUNCTION APPROXIMATION BY SYNTHETIC GRN

In this section we address the demonstration that finite feed-forward networks of Hill equations can approximate any positive, bounded, continuous function defined over a bounded subset of the real numbers: such systems already incorporate the needed non-linearities that linear perceptrons were missing. For the demonstration we use only Hill equations at their steady state, which already encompass enough complexity for our purposes. This work shall just be a glance into the actual capabilities of GRNs if we would use them as computing devices. Incorporating feedback or analyzing the evolution in time would reveal GRNs as appropriate substrates to biologically implement more powerful devices such as recurrent or echo state networks (Maass et al. 2002).

A. Mathematical characterization of Hill functions

Hill differential equations model the temporal dynamics of the concentration $y(t)$ of a protein $Y$ regulated by a set of $N$ promoter proteins $X_i$ whose concentrations are $x_i(t)$. In the literature we can find different implementations of these dynamics depending on whether or not different promoters can associate with each other to express $Y$ (Hasty et al. 2001; Goutelle 2008; Santillán 2008). We choose a formalism in which such cooperation is banned:

$$\frac{dy}{dt} = \frac{\alpha_0 + \alpha_1 x_1^{n_1} + \ldots + \alpha_N x_N^{n_N}}{\beta_0 + \beta_1 x_1^{m_1} + \ldots + \beta_N x_N^{m_N}} - \kappa y. \quad (1)$$

Here $\kappa$ represents the degradation rate of $Y$, $n_i$ with $i = 1, \ldots, N$ are Hill coefficients that estimate the number of
Consider an all-or-none response (red line) but real systems follow a smooth profile (dashed line) that can be characterized by means of Hill functions.

If the regulating agents is present.

The responses are often sharp, reminding us the ones observed in neural systems. In response of a given element (\( S_i \)) requires a total input reaching a value higher than a critical threshold \( \theta_i \).

We will see now how a clever use of such a simple motif is enough to show the very rich computational capabilities of networks of GRNs. We begin our study by a thorough characterization of the steady state of this equation, part of which might already be found in the literature.

For a fixed concentration \( x \) of protein \( X \), equation (2) reduces towards:

\[
\frac{dy}{dt} = \frac{\alpha_0 + \alpha_1 x^n}{\beta_0 + \beta_1 x^n} - \kappa y.
\]

Let us note that a modeling allowing a cooperative action of different \( X_i \) upon \( Y \) also reduces to this expression for just one regulating protein, thus the following results are general.

Let us note that \((\alpha_1 \beta_0 - \beta_1 \alpha_0)\) determines the sign of this slope and that this sign remains unchanged for the

**FIG. 1 Information processing in living cells** Cells are complex living computational devices. The upper diagram (picture adapted from Lim 2010) is a simplified drawing including some basic components of the computational logic of cells. In this simplified description, cells gather signals of different nature from the external world (while sensing their internal state) and respond to these signals by means of information-processing networks, eventually triggering biochemical and physical changes as output. a Gene networks are one type of such molecular machinery. Here we represent their expression and response to DNA-binding proteins (so called transcription factors) using arrows to indicate who influences whom and weights \( W_i \) to represent the strength of the regulatory interaction. A formal approach to the same network is shown in b where genes are formally represented as state variables in a nonlinear dynamical system (see text). The responses are often sharp, reminding us the ones observed in neural systems. In c an example of the type of nonlinearity considered here is shown. Here the activation response of a given element (\( S_3 \)) requires a total input reaching a value higher than a critical threshold \( \theta_3 \). Idealized models consider a all-or-none response (red line) but real systems follow a smooth profile (dashed line) that can be characterized by means of Hill functions.

\( X_i \) molecules required for a functional effect on \( Y \), \( \alpha_i \) and \( \beta_i \) indicate the affinity of \( Y \) to each one of the regulating proteins, and \( \alpha_0 \) and \( \beta_0 \) encode for the basal activity of \( Y \) (i.e. the concentration of that protein when none of the regulating agents is present).

Other formulations of the sought dynamics are qualitatively similar regarding the task that we have ahead. If \( Y \) is regulated by just one promoter \( X \) with concentration \( x \), equation (1) reduces to:

\[
\frac{dy}{dt} = \frac{\alpha_0 + \alpha_1 x^n}{\beta_0 + \beta_1 x^n} - \kappa y.
\]

Let us note that a modeling allowing a cooperative action of different \( X_i \) upon \( Y \) also reduces to this expression for just one regulating protein, thus the following results are general.

We will see now how a clever use of such a simple motif is enough to show the very rich computational capabilities of networks of GRNs. We begin our study by a thorough characterization of the steady state of this equation, part of which might already be found in the literature.

For a fixed concentration \( x \) of protein \( X \), equation (2) reduces towards:

\[
\tilde{y}(x) = \frac{\alpha_0 + \alpha_1 x^n}{\kappa [\beta_0 + \beta_1 x^n]}.
\]

\( \tilde{y}(x) \) representing the concentration of \( Y \) at the steady state as a function of the concentration of its promoter. The slope of this function with respect to \( x \) reads:

\[
\frac{d\tilde{y}(x)}{dx} = \frac{n(\alpha_1 \beta_0 - \beta_1 \alpha_0) x^{n-1}}{\kappa [\beta_0 + \beta_1 x^n]^2} \equiv D_\gamma(x).
\]

Let us note that \((\alpha_1 \beta_0 - \beta_1 \alpha_0)\) determines the sign of this slope and that this sign remains unchanged for the.
whole domain of $\tilde{g}(x)$, meaning that this function is either monotonously increasing or decreasing.

If we calculate the second derivative of $\tilde{g}(x)$:

$$\frac{d^2\tilde{g}(x)}{dx^2} = \frac{d^2\tilde{g}(x)}{dx^2} = n(\alpha_1\beta_0 - \beta_1\alpha_0)x^{n-2} \times \frac{(n-1)\beta_0 - (n+1)\beta_1x^n}{\kappa [\beta_0 + \beta_1x^n]^3}, \quad (5)$$

and calculate the extrema of $D\tilde{g}(x)$, $\frac{dD\tilde{g}(x)}{dx} = 0$; we find three different solutions:

- $x^{n-2} = 0 \iff x = 0 \equiv x_0, \ n > 2$.
- $\frac{1}{|\beta_0 + \beta_1x^n|} \to 0 \iff x \to +\infty \equiv x_\infty$.
- $(n-1)\beta_0 - (n+1)\beta_1x^n = 0 \iff x = \left[\frac{(n-1)\beta_0}{(n+1)\beta_1}\right]^{1/n} \equiv x_\theta$.

The last extremum of $D\tilde{g}(x)$ is the most interesting one as it indicates an inflexion point of the original function $\tilde{g}(x)$. For convenience and similarity to sigmoids, we will call $x = x_\theta$ the threshold of the Hill equation – thus the notation. We evaluate the slope at the threshold:

$$D\tilde{g}(x_\theta) = \frac{(n+1)^2(\alpha_1\beta_0 - \beta_1\alpha_0)}{4n\kappa\beta_0^n} \left[\frac{(n-1)\beta_0}{(n+1)\beta_1}\right]^{n-1} \quad (6)$$

By now we have got our Hill equation characterized by parameters that are rather abstract from a geometric point of view, although their biological meaning is clear. Let us write $\alpha_0$, $\alpha_1$, $\beta_0$, and $\beta_1$ in terms of $\tilde{g}(0)$, $\tilde{g}(\infty)$, and $x_\theta$; which have got a more intuitive geometric interpretation. For the upper and lower limits of $\tilde{g}(x)$ we get:

$$\lim_{x \to 0^+} \tilde{g}(x) = \frac{\alpha_0}{\kappa\beta_0} \equiv \tilde{g}(0) \Rightarrow \alpha_0 = \kappa\beta_0\tilde{g}(0).$$

$$\lim_{x \to +\infty} \tilde{g}(x) = \frac{\alpha_1}{\kappa\beta_1} \equiv \tilde{g}(\infty) \Rightarrow \alpha_1 = \kappa\beta_1\tilde{g}(\infty). \quad (7)$$

From the equation for $x_\theta$ we can work out the ratio $\beta_0/\beta_1$:

$$\frac{\beta_0}{\beta_1} = \frac{n+1}{n-1}x_\theta^n, \quad (8)$$

and substituting in equation (6) we can write the slope of $\tilde{g}(x)$ at the threshold as a function of the desired parameters:

$$D\tilde{g}(x_\theta) = \left[\tilde{g}(\infty) - \tilde{g}(0)\right] \frac{(n-1)(n+1)}{4n}x_\theta^{n-2}. \quad (9)$$

It is clear that $\tilde{g}(0)$, $\tilde{g}(\infty)$, and $x_\theta$ should be given if we wanted to build up a Hill function with customized upper and lower bounds and threshold. It would only remain $n$ to control the steepness given by equation (9). Indeed:

$$\lim_{n \to 1^+} ||D\tilde{g}(x_\theta)|| = 0, \quad \lim_{n \to +\infty} ||D\tilde{g}(x_\theta)|| = +\infty; \quad (10)$$

while $[\tilde{g}(\infty) - \tilde{g}(0)]$ still controls the sign of the bare derivative. This indicates that varying $n$ we can design Hill equations that are more or less flat. This also makes manifest the role of a large $n$ to introduce non-linearities in protein regulation processes, as well as its importance for sharply triggered dynamics.

Let us note that we went from a parameterization using $\kappa$, $n$, $\alpha_0$, $\alpha_1$, $\beta_0$, and $\beta_1$ to one that only uses $n$, $\tilde{g}(0)$, $\tilde{g}(\infty)$, and $x_\theta$. This is possible because $\kappa$ plays a normalizing role and can be absorbed into some other constant. Also, we saw that $\beta_0$ and $\beta_1$ only affect the shape of the Hill equations through their ratio.

Now, by choosing $\tilde{g}(0)$ and $\tilde{g}(\infty)$ we can build a Hill function with any wished upper and lower bounds, and choosing $x_\theta$ and $n$ we can set up any desired threshold and steepness. From these choices we could work out the values of the original set of parameters and we would still have freedom to choose $\kappa$ and either $\beta_0$ or $\beta_1$.

A key ingredient in our demonstration will be that we can approximate a step function with any desired accuracy. This can be done taking $n \to \infty$, and this would be completely fair from a mathematical point of view. However, our endeavor is to show the computational capacity of realistic biological systems. Regulatory systems such as those described by equation (2) usually present low values of $n$. A typical value is $n \approx 2$, which is consistent with molecular processes based on dimerization (Alon 2006, Macía et al. 2009). Luckily enough, there is a very interesting way around to approximate a step function by combining Hill equations; and one that the nature itself seems to have used prominently for threshold-dynamics regulation. This is explored in the following subsection.

B. Cascades of Hill equations

An interesting feature of sigmoid functions is that they map an interval (let us say $x \in (0, +\infty)$) into another (say $y \in (0, +1)$) in an exponential fashion, meaning that a linear increase in $x$ corresponds to a somehow exponential modification of $y$. This can be seen in the exponential decay of $y$ towards 0 or 1 when $x \to 0$ or $x \to +\infty$ and in the exponential increase of $y(x)$ when $x$ approaches the threshold of the sigmoid. If this characteristic is also true for Hill functions we can use the output of one such equation as the input of another with the hope of transiting exponentially faster through the threshold of the second equation when varying the original variable, thus providing a steepest overall dependency. This turns out to be the case as it is demonstrated in figures 2a and 2b.
Using combinations of Hill equations, in this subsection we aim directly at building a step function and a piecewise defined function that is constant and different from 0 for a range of x, and 0 out of this range – i.e. a rectangular function \( R(x) \). In doing so we will disregard other interesting functions that combinations of Hill equations might be producing. In this line, we begin by calling strictly excitatory \( \tilde{g}^E(x) \) and strictly inhibitory \( \tilde{g}^I(x) \) Hill equations to those with \( \tilde{g}^E(0) = 0 \), \( \tilde{g}^E(\infty) > 0 \) and \( \tilde{g}^I(0) > 0 \), \( \tilde{g}^I(\infty) = 0 \) respectively.

We define a \( M \)-Cascade function – and note it \( C_M(x) \) – as the system of \( M \) Hill equations coupled such that:

\[
C_M(x) = \tilde{y}_M \left(C_{M-1}(x), \right),
\]

\[
C_1(x) = \tilde{y}_1(x),
\]

This system can have very rich dynamics depending on the set \( \{\tilde{g}_m(x); m = 1, ..., M\} \) of Hill equations used, which might be inhibitory or excitatory; but for our im-

---

**FIG. 2** Approximating a rectangular function with superpositions of Hill equations. a A superposition of excitatory Hill equations \( \tilde{g}_m^E(x) \), with \( m = 1, ..., 7 \) builds up an excitatory cascade \( C^E_M(x) \) that approaches an excitatory step function. b An inhibitory Hill equation \( \tilde{g}_m^I(x) \) is combined with a superposition of excitatory Hill equations \( \tilde{g}_m^E(x) \), with \( m = 2, ..., 7 \) to build up an inhibitory cascade \( C^I_M(x) \) that approaches an inhibitory step function. \( \tilde{g}^E(x) \) in a and \( \tilde{g}^I(x) \) in b are plotted with a continuous black line, and in both a and b \( \tilde{g}_m^E(x) \) is plotted with a continuous red line. Intermediate equations \( \tilde{g}_m^E(x); m = 2, ..., 6 \) are always plotted with dashed lines. Steepness grows with \( m \). The upper limit of the Hill equations has been set such that all excitatory units take values approximately between 0 and 1. Therefore, the inhibitory unit in b had to be designed with a \( \tilde{g}_1^I(0) > 1, 0 < \tilde{g}_1^I(\infty) < \tilde{g}_1^I(0) \); so it is not a strictly excitatory unit as they were defined on the text. c Excitatory \( C^E_{M'}(x) \) (black) and inhibitory \( C^I_M(x) \) (red) cascades fulfilling the necessary relationships to build a rectangular function. Back to the biological interpretation of our model, we must assume that both cascades are different metabolic pathways promoted by a same protein \( X \) that presents a concentration \( x \) working as the input of the whole system. Both pathways would produce a same protein \( X^E_2 \), whose joint yield as a function of \( x \) is shown in panel d. A third cascade must be added, \( C^E_{M''}(x) \); an excitatory one, and its threshold (horizontal red line in panel d) must be appropriately chosen to approach a rectangular function \( R(x) \). e Tuning \( C^E_{M''}(\infty) \) we can approximate the desired \( R(x) \): A rectangular function with \( R(x) = 1 \) if \( x \in [0.5, 1.5) \) and 0 elsewhere is faithfully approximated by \( I(x) \equiv C^E_{M''}(x) \) (black).
mediate purpose (approaching a step function) we are just interested in a set of equations such that all of them transit through their threshold at the same time, thus making sure that \( C_M(x) \) has got a well defined threshold \( x_\theta \) itself. To do this, we must build the \( y_m(x) \) such that \( x_{\theta,m} = y_{m-1}(x_{\theta,m-1}) \) for all \( m \) except \( x_{\theta,1} \equiv x_{C_M} \), which still remains free for us to choose. This is always possible for any \( x_{C_M} > 0 \), as we saw in the previous subsection.

We can construct an excitatory cascade \( C_E^M(x) \): a cascade with \( C_E^M(0) = 0 \) and \( C_E^M(\infty) > 0 \). This is done by piling up a set of excitatory Hill equations \( \{ y_m^E(x); m = 1, \ldots, M \} \), as shown in figure 2a. We can also build an inhibitory cascade \( C_I^M(x) \): a cascade with \( C_I^M(0) > 0 \) and \( C_I^M(\infty) = 0 \) in the same way as the excitatory one, but now an odd number of inhibitory Hill functions must be used together with an arbitrary number of excitatory units. For simplicity we have built our inhibitory cascades with the first Hill equation of the cascade being inhibitory and the remaining ones being excitatory \( \{ y_m^E(x); m = 2, \ldots, M \} \). One such a cascade is shown in figure 2b.

In both excitatory and inhibitory cascades a problem comes up regarding their upper or lower limits. The first function in the cascade (we shall be using just excitatory units in this paragraph without loss of generality) is defined for \( x_1 \in [0, +\infty) \) and takes values over \( y_1^E \in [0, y_1^E(\infty) < +\infty) \). When feeding \( y_1^E(x) \) as an input for the following unit, this second equation will have an input \( x_2 = y_1^E \in [0, y_1^E(\infty) < +\infty) \), meaning that it will take values \( y_2^E \in [0, y_2^E(y_1^E(\infty)) < y_2^E(\infty)) \).

We can see that the last equation of the cascade will never reach its upper limit because its input does not span the whole domain of positive real numbers, so if we wanted to build our cascade such that it would have the \( C_M(\infty) \) we desire, we would have to set the upper bound of the last equation of the cascade to be \( y_M(\infty) = \gamma C_M(\infty) \), where \( \gamma \) is a positive constant. This is always possible since \( y_M(\infty) \) is allowed to take any positive value we desire. The same reasoning applies for inhibitory cascades. In figures 2a and 2b, the \( y_M(\infty) \) of all equations were corrected such that the cascades have a maximum of roughly 1. We note that we have got a relative freedom to choose most of \( y_m(0) \) and \( y_m(\infty) \).

Using an inhibitory cascade \( C_{I_1}^M(x_{I_1} = x) \) and two excitatory ones \( C_{E_1}^M(x_{E_1} = x) \), \( C_{E_2}^M(x_{E_2} = C_{E_1}^M + C_{E_2}^M) \), we can build up an interesting mathematical device. Let these units be such that:

\[
\begin{align*}
  x_{\theta}^E &< x_{\theta}^I, \\
  C_{I_1}^M(0) &< x_{\theta}^E < C_{I_1}^M(0) + C_{E_1}^M(\infty), \\
  C_{E_2}^M(\infty) &< x_{\theta}^E.
\end{align*}
\]

(12)

The behavior of a couple of cascades \( C_{I_1}^M(x) \) and \( C_{E_2}^M(x) \) fulfilling these relationships is plotted in figure 2b:

\( C_{E_2}^M(x_{E_2}) \) is slightly different from all the functions used so far. It would not, in principle, be exactly described by equation 2 because its input is a sum of terms, i.e. it would not be regulated by just one promoter. However, using the idea of distributed computation (Regot et al. 2011) we could easily envision a case whose input is the sum of two independent terms while it can still be modeled by the equation that we have so thoroughly described: \( C_{I_1}^M(x) \) and \( C_{E_2}^M(x) \) must constitute two alternative pathways for the production of a same protein \( X_{E_2} \), such that the concentration of this protein would be the total yield, as required: \( x_{E_2} = C_{I_1}^M + C_{E_2}^M \). Assuming that \( C_{I_1}^M \) and \( C_{E_2}^M \) are exactly this kind of cascades, the total yield of protein \( X_{E_2} \) as a function of \( x \) is shown in figure 2b together with a red line that indicates the threshold \( x_{\theta}^E \) of the third cascade.

Let us note that the threshold of the third cascade \( C_{E_2}^M(x_{E_2}) \) is reached only when the two former are activated at the same time, thus serving as an AND logic gate. But the interest of this object goes beyond the AND gate. Let us call \( I(x) \equiv C_{E_2}^M(x) \) to the exact function implemented by such an assembly of Hill equations as a function of \( x \) (figure 2b). \( I(x) \) can approach a rectangular function \( R(x) \) with any desired accuracy. We just need to adjust \( C_{E_2}^M(\infty) \) to the height of \( R(x) \) and the corresponding thresholds \( x_{\theta}^E \equiv x_{\theta}^E \) and \( x_{\theta}^I \equiv x_{\theta}^I \) to match those of \( R(x) \). A steepen enough transition through the threshold is achieved, as we already know, by cascading a sufficient number \( M, M', M'' \) of Hill equations.

C. Feed-forward GRN as Function Approximators

With the elements built so far we are ripe to show that we can approximate any bounded, positive, continuous function of \( N_0 \) real, positive variables with a finite feed forward network of Hill equations.

For simplicity we proceed for \( N_0 = 1 \), without loss of generality. Let there be \( f(x) \) a continuous function taking bounded real, positive values and defined over a finitely bounded subset of real, positive numbers. We can always find a finite partition \( X_r = \{ x_{r,i}; i = 1, \ldots, n \} \) such that for all \( i = 1, \ldots, n \) it does not exist any \( x_{r,i} \in [x_{r,i}, x_{r,i+1}] \) such that \( f(x_{r,i}) > f(x_{r,i}) + \epsilon \) and it does not exist any \( x_{r,i} \in [x_{r,i}, x_{r,i+1}] \) such that \( f(x_{r,i}) < f(x_{r,i}) - \epsilon \) for any \( \epsilon > 0 \).

We can find a finite collection of Hill equations with which it is possible to build a set \( I_r = \{ I_{r,i}(x); i = 1, \ldots, n \} \) of functions such that \( I_{r,i}(x) \approx f(x_{r,i}) \), if \( x \in [x_{r,i}, x_{r,i+1}] \) and \( I_{r,i}(x) \approx 0 \) otherwise. Then the function:

\[
F_r(x) = \sum_{i=1}^{n-1} I_{r,i}(x)
\]

(13)

approximates \( f(x) \) with an error approximately lower than \( \epsilon \).

To integrate the different outcomes of the collection \( I_r \) into one single output function \( F_r \), it might seem necessary a modeling with several promoters. We would be
FIG. 3 A GRN model is trained to fit the function \( t(x) = \sin(2\pi \nu x) + 2 \). A three-layered architecture with 4-4-1 units at input, hidden and output layers respectively was chosen. Units at the input layer are regulated by one external promoter whose concentration acts as input to the whole system and to the target function. Results are shown for different values of \( \nu \): a continuous black curve indicates the actual target function while red crosses indicate the outcome of the trained GRN at a given input concentration \( x \). a \( \nu = 1 \): the easiest task possible. b \( \nu = 2 \): a slightly more complicated case, but the GRN still performs quite adequately. c \( \nu = 3 \): as the difficulty increases, the network can not respond as faithfully as desired anymore. d \( \nu = 4 \): for very complicated instances, two aspects contribute to distort the output: on the one hand, a larger network might be needed to reproduce very intricate functions; on the other hand, GRNs are trained using a finite sampling of \( t(x) \), which can only give a hint of the real complexity of the target function.

III. BACK-PROPAGATION ALGORITHM FOR GRN

Lacking an algorithm to train multilayered artificial neural networks in solving specific tasks was an important drawback that prevented notable advances in AI for around two decades (Olazaran 1989). Training a network means finding a set of parameters for its constituents such that the network as a whole behaves in a desired way. Training – or learning – methods are classified as supervised when examples of the expected behavior are provided or unsupervised if a network is expected to work out the structure of a problem on its own.

The most successful method of supervised learning is the back-propagation algorithm (Rumelhart et al. 1986) – a generalization to complex structures of error minimization by hill climbing that is possible thanks to a nimble handling of partial derivatives and the chain rule. Notwithstanding this, our networks of Hill equations
present several redundancies and particularities and some choices had to be made to implement a working algorithm. We did so, and the proof that our choices take on the training can be found in section IV where we apply our methods to four basic machine learning problems. However, discussion remains open about how correct some of our decisions are. Several alternatives might be equally right, and further research should be made to tell the most efficient learning strategies for synthetic GRNs.

We will be working with an arbitrary feed-forward network of Hill equations. The network consists of a series of units, of Hill equations arranged in \( L \) layers. Each layer is labeled with the superindex \( j \) and has got \( N_j \) units in it. The first layer will be called the input layer and the last one will be called the output layer. Such a network is a model for a GRN of promoter proteins that regulate each other in a feed-forward manner such that proteins regulated at layer \( j \) act as promoters of those regulated at layer \( j+1 \). The concentration of each unit is governed by a formula similar to equation (9). We will rewrite this equation right ahead and therefore we shall adopt a new notation that exhaustively uses sub- and superindexes. The notation may seem baroque but it is a very appropriate one to derive back-propagation rules.

We call \( X_j^i \) to the \( i \)-th protein in the \( j \)-th layer, and \( x_i^j \) to its concentration. We write the differential Hill equation for the time evolution of this concentration:

\[
\frac{dx_i^j}{dt} = \frac{\alpha_i^{j,0} + \sum_{i'=1}^{N_j} \alpha_i^{j,i'}(x_i^{j'})^n}{\beta_i^{j,0} + \sum_{i'=1}^{N_j} \beta_i^{j,i'}(x_i^{j'})^n} - \kappa_i^j x_i^j. \tag{14}
\]

Here we have just added a collection of sub- and superindexes to equation \( \ref{eq:hill} \) and collapsed the sums within the corresponding symbols, but both equations are essentially the same. The only actual difference is that we assumed just one and the same Hill coefficient \( n \) for all the proteins in the network. Regarding the notation, we use \( j' \) to name the layer whose output works as input for layer \( j \). This usually means \( j' = j-1 \), but for the input layer we must assume that there is an extra layer \( j' = 0 \) consisting of the promoters that act as input for the whole system.

We make this notation even more compact with a classic trick in machine learning: assuming that there exist several external units with constant concentration \( x_j^0 = 1 \). Then: \( \alpha_i^{j,0} \to \alpha_i^{j} \) and \( \beta_i^{j,0} \to \beta_i^{j} \). Also, \( \kappa_i^j \) can be integrated into other constants by doing: \( \frac{\alpha_i^{j,i'} \to \tilde{\alpha}_i^{j,i'}}{\kappa_i^j} \). Equation \( \ref{eq:hill} \) is rewritten as:

\[
\frac{1}{\kappa_i^j} \frac{dx_i^j}{dt} = \frac{\sum_{i'=0}^{N_j} \tilde{\alpha}_i^{j,i'}(x_i^{j'})^n}{\sum_{i'=0}^{N_j} \beta_i^{j,i'}(x_i^{j'})^n} - x_i^j, \tag{15}
\]

such that the concentration of protein \( X_j^i \) when the network reaches a steady state is:

\[
x_i^j = \frac{\sum_{i'=0}^{N_j} \tilde{\alpha}_i^{j,i'}(x_i^{j'})^n}{\sum_{i'=0}^{N_j} \beta_i^{j,i'}(x_i^{j'})^n}. \tag{16}
\]

The set of equations for the whole network successively determines the concentration of proteins at each layer once the concentration of the promoters of the input layer is given. We read out the concentration of proteins in the output layer as a sort of result of the computation that the GRN implements. We see that the network computes in a feed-forward manner. At the training phase, this outcome is compared to some target function that encompasses the behavior that is expected from the network. This will be clearer in section IV when examples are shown. By now it is enough to assume that an error can be derived by comparing the target function to the result yielded by the network. This error will be propagated backwards throughout the network, and the parameters \( \tilde{\alpha}_i^{j,i'} \) and \( \beta_i^{j,i'} \) will be modified according to how much of the error each of them is responsible of. To implement this back-propagation concept, the following derivatives will be necessary:

\[
\frac{\partial x_i^j}{\partial \tilde{\alpha}_i^{j,i'}} = \frac{(x_i^{j'})^n}{\sum_{i'=0}^{N_j} \beta_i^{j,i'}(x_i^{j'})^n}, \quad \frac{\partial x_i^j}{\partial \beta_i^{j,i'}} = -x_i^j \frac{(x_i^{j'})^n}{\sum_{i'=0}^{N_j} \beta_i^{j,i'}(x_i^{j'})^n}, \quad \frac{\partial x_i^j}{\partial \tilde{\alpha}_i^{j,i'}} = n \frac{x_i^j}{x_i^{j'}} \left[ \frac{\partial \tilde{x}_i^{j,i'}}{\partial \tilde{\alpha}_i^{j,i'}} + \frac{\partial \tilde{x}_i^{j,i'}}{\partial \beta_i^{j,i'}} \cdot \frac{\partial \beta_i^{j,i'}}{\partial \tilde{\alpha}_i^{j,i'}} \right]. \tag{17}
\]

The next step is to make use of the error function \( e(x_i^{N_L}; t_i) \) whose existence we just assumed. This depends on the concentrations \( x_i^{N_L} \) at the output layer and on a target function \( T \equiv \{ t_i \} \) that has got as many components as units there are in the output layer. Let us note that both \( x_i^{N_L} \) and \( T \) are a function of the concentration of the promoters of the input layer. We define the partial error:

\[
\delta_i^j = -\frac{\partial e(x_i^{N_L}; T)}{\partial x_i^j}. \tag{18}
\]

of the \( i \)-th unit in layer \( j \). For the output layer the partial error can be obtained straight away by taking derivatives in the error function. For other layers it is necessary to use the chain rule as it follows:

\[
\delta_i^j = -\frac{\partial e(x_i^{N_L}; T)}{\partial x_i^j} = \sum_{i=1}^{N_j} \left( \frac{\partial e}{\partial x_i^j} \cdot \frac{\partial x_i^j}{\partial x_i^{j'}} \right) = \sum_{i=1}^{N_j} \delta_i^{j'} \cdot \frac{\partial x_i^j}{\partial x_i^{j'}}. \tag{19}
\]
This allows us to compute the error of units at layer $j'$ once the errors in $j$ are known, thus back-propagation. Knowing these partial errors at each unit it is easy to calculate the gradient of the error with respect to the different parameters of the network $\tilde{\alpha}_{ik}^{j'}$ and $\tilde{\beta}_{ik}^{j'}$, and apply a hill climbing rule on them:

$$
\Delta \tilde{\alpha}_{ik}^{j'} = -\eta \cdot \frac{\partial \epsilon (x^{out}, T)}{\partial \tilde{\alpha}_{ik}^{j'}} = -\eta \cdot \frac{\partial \epsilon}{\partial x_i} \frac{\partial x_i}{\partial \tilde{\alpha}_{ik}^{j'}} = -\eta \tilde{\alpha}_{ik}^{j'} \frac{\partial x_i}{\partial \alpha_{ik}^{j'}} ,
$$

$$
\Delta \tilde{\beta}_{ik}^{j'} = -\eta \cdot \frac{\partial \epsilon (x^{out}, T)}{\partial \tilde{\beta}_{ik}^{j'}} = -\eta \cdot \frac{\partial \epsilon}{\partial x_i} \frac{\partial x_i}{\partial \tilde{\beta}_{ik}^{j'}} = -\eta \tilde{\beta}_{ik}^{j'} \frac{\partial x_i}{\partial \beta_{ik}^{j'}} ,
$$

where $\eta$ represents a learning rate whose value has to be chosen externally for each training task, and $\Delta \tilde{\alpha}_{ik}^{j'} + \frac{\partial \epsilon}{\partial \tilde{\alpha}_{ik}^{j'}}$, and $\Delta \tilde{\beta}_{ik}^{j'} + \frac{\partial \epsilon}{\partial \tilde{\beta}_{ik}^{j'}}$, are the update rules for each parameter at each training step.

We note straightaway that such an update rule can lead to negative values of $\tilde{\alpha}_{ik}^{j'}$ or $\tilde{\beta}_{ik}^{j'}$, which we would like to avoid because these constants are always positive.

With this we are ready to go ahead with some easy machine learning examples. The back-propagation rules derived here are just a straightforward implementation of the standard algorithm applied to ANNs, with the only difference that each pair of units is linked by two weights. Apart from this, more modern implementations could be done that incorporate dynamic tuning of $\eta$ or memory effects from the training history, or that exploit some stochasticity to avoid converging towards local minima. Such improvements would speed up the training or guarantee that the desired behavior would be better reproduced. We are not concerned with these issues now:

\[\text{FIG. 4 Classification problems.} \quad \text{Two inputs} \ x \ \text{and} \ y \ \text{represent the concentrations of the regulating input proteins} \ X \ \text{and} \ Y. \ \text{They can also be plotted as two-dimensional coordinates. Given a point} \ (x, y) \ \text{it was considered that the GRN classified the coordinate as class 1 if} \ x^\text{out} > x^\text{out}_0 \ \text{and as class 2 otherwise. Class 1 points are plotted in red and class 2 are plotted in green. The GNRs were tested with the coordinates of a regular grid after their training. Along with the GRN’s outcome for the grid, it is plotted the set of training points: class 1 samples as blue crosses and class 2 samples as black squares. The training set was generated with some noise, such that the classes were not separable with simple geometrical shapes.} \ a \ \text{The circle turned out to be the one requiring a less complex network to be solved, a 4-4-2 architecture.} \ b \ \text{The XOR needed 5-10-2 units in each layer. This seems exaggerated for such a seemingly simple problem. Presumably a smaller network could be reached if additional wiring-optimization algorithms were used.} \ c \ \text{The spiral pattern is a difficult one and it required 10-10-2 units to achieve the shown results.}\]
this work intends to demonstrate the concept of machine learning implemented on models of GRNs. Because of this, it will be found that examples from the following section require an exaggerated training period (for nowadays machine learning standards), or that convergence could be better than shown. It should be kept in mind that there is still plenty of room for improving the presented methods.

IV. FOUR EASY MACHINE LEARNING TASKS

As a proof of concept, we used feed-forward networks of Hill equations and the proposed training rules to solve four easy machine learning problems: one fitting and three classification tasks.

During the learning phase we appreciated that our algorithm is quite sensitive to initial conditions. The initial values of $\alpha_{ij}^{\prime}$ and $\beta_{ij}^{\prime}$ are chosen randomly before learning, and an unlucky initialization can harm convergence towards the desired network, probably because the algorithm gets stuck in local minima. This is common also in very basic ANNs implementations.

We were not specially interested in constructing optimal GRNs (meaning GRNs with less units or connections and yet with a good performance). Network growing or pruning algorithms could be implemented for this end. Our main interest for this paper was to show some working examples of the ideas developed, and thus we proceeded using the most basic methods.

Because some freedom remained to set up the $\kappa_{j}$ and $n$ we chose $n = 2$, which is a very realistic value for systems modeled by Hill equations, and $\kappa = 1$. This applies to all the examples following.

A. Using GRNs to fit a function

In the first task we wanted our network to behave such that when an input $x$ was given to the input layer, then the output layer would yield $t(x)$, where $t(x)$ is an arbitrary mathematical function that we shall call the target of our training. In biological terms, the proteins at the input layer of our GRN would be exposed to a concentration $x$ of their promoter protein $X$, and it would be wished that the concentration $x_{\text{out}}$ of the protein $X_{\text{out}}$ at the output layer would be exactly $t(x)$.

We used $t(x) = \sin(2\pi \nu x) + 2$ with $\nu = 1, 2, 3$, and $4$ and $x \in [0, 1)$. This is a function of only one variable, thus proteins in the input layer would be controlled by just one external promoter; it is always positive, as required in section II for minimally realistic systems; and it is unidimensional, thus one only unit is required in the output layer.

Many different architectures were tried, but the results shown here correspond to networks with three layers: the input one with 4 units, a hidden layer with 4 more units, and the output layer. We used the error function:

$$e(x_{\text{out}}; T) = \frac{(x_{\text{out}} - t(x) + \xi)^2}{2},$$

(23)

where $\xi$ is a Gaussian noise with 0 mean and standard deviation 0.1, so that perfect examples did probably not show up.
For the training, 100 data points \((x, t(x))\) were generated and each point was presented 1000 times to the GRN. The back-propagation algorithm was applied right after each data presentation. Results for the different \(\nu\) are shown in figure 3. We appreciate how the performance gets worst as the complexity of the function within \([0, 1]\) increases. Apart from the obvious need for more units to reconstruct finer details, we must concede that the training points do not need to reveal the whole structure of the target function because they are just a noisy, finite sample of it.

### B. Three classification tasks

We made up three classification tasks of varying difficulty. For each task we generated 1000 data points belonging to classes 1 or 2. Each point consists of a two-dimensional coordinate and a label is attached that indicates its class. The different classes are arranged on a plane. We identify each task as circle, XOR, and spiral after the geometric disposition of the data (see figure 4). Noise was added such that the classes overlap and they are not easily separable. These points were used to train the network: back-propagation was applied right after the presentation of each data point and each point was presented 100 times. When the learning phase was over, a grid that covered the area spanned by the training samples was given to the network to test its performance.

The input now is a two-dimensional coordinate, thus proteins of the input layer have got two external promoters \(X\) and \(Y\) with concentrations \(x\) and \(y\). It would have been possible to read out the result with just one output unit, attending to whether its concentration was above or below an arbitrary threshold. It was decided, though, to always use two units \(X_1^{\text{out}}\) and \(X_2^{\text{out}}\) in the output layer and interpret the result as class 1 if \(x_1^{\text{out}} > x_2^{\text{out}}\) and as class 2 otherwise. It was used the following error function:

\[
\epsilon(x^{\text{out}}; T) = \frac{(x_1^{\text{out}} - t_1)^2 + (x_2^{\text{out}} - t_2)^2}{2},
\]

where \(T = (1, 0)\) if the input belonged to class 1 and \(T = (0, 1)\) if it belonged to class 2.

The circle task (figure 4a) happened to be the less demanding one: it was solved with just 3 units in the input layer and 2 units in the hidden layer, plus two output units as said before. The network obtained is represented in figure 5. This reminds us of the small feed-forward network motifs from real cells (figure 2).

Because the tasks were of growing difficulty, the number of units needed to solve each problem changed. The XOR (figure 4b) required 5 and 10 units in the input and hidden layers, and the spiral (figure 4c) could not be solved with less than 10 units in each non-output layer. Both of them had 2 units as an output.

Let us recall once more that it was not sought any optimization in terms of wiring or number of nodes. It can be expected that much smaller GRNs can be designed to solve either of these problems (including also the function fitting task) if network growing or pruning algorithms were employed. Such methods are quite common in ANNs architecture optimization, which is a non-trivial problem. Taking into account that we did not care about the wiring that much, the results obtained are very satisfactory.

### V. DISCUSSION

Within the developing framework of biological computation, in this paper we proposed that feed-forward GRNs are a suitable substrate to implement the basics of machine learning. They can cope perfectly with solving regression problems or with data classification, as it has been explicitly shown in four examples. This opens the door to more complicated AI applications such as data mining, data series prediction, clustering, signal filtering, etc. Of course, using networks of Hill equations does not suppose an improvement in performance when compared to existing ANNs. This was not the aim of this work. Our purpose was rather to show that realistic biological systems have got a complexity enough as to solve machine learning tasks. We did so with a constructive – enough, but not completely rigorous – mathematical proof that the chosen GRN model can approximate any bounded, positive, continuous function and presenting four practical examples.

GRNs appear as a quite adequate means to implement ANNs-like architectures. Already in cellular systems, genes appear arranged in networks and execute very complex duties. Because of this network disposition, the models we work with are readily suitable to derive error back-propagation rules. This is the most successful algorithm for training ANNs in implementing arbitrary functions. It is an analytical tool, a theoretical device; so knowing these rules for networks of Hill equations we can design ideal GRNs that would behave as we wished.

Of course, back-propagation does not provide a technique to build synthetic networks in vivo, but rather the instructions for what will be needed when the moment arrives. With the idea of providing a faithful, realistic plan, there are a series of constraints that we should sooner or later impose in the large set of parameters that our GRNs would have. For the present paper we just demanded that our networks should always have positive affinities and positive basal expression levels, and that Hill coefficients should be low enough. For the practical examples we used \(n = 2\), which is among the lowest possible. Also, in these examples we did not care that much if the values of certain parameters turned out exageratedly huge or small after training – as it was the case, among other reasons because we have been working with equations in arbitrary dimensions. But requesting that these parameters fall within a physically plausible range and many other necessary constraints can be easily
incorporated into the back-propagation rules so that we would arrive to feasible GRNs.

An immediate critique to the idea introduced in this paper is that its development in actual biological systems may require a very fine-tuning of parameters that is out of reach for state-of-the-art experimental techniques. The scenario might be even worst: since stochasticity is so in the core of molecular biology, narrowly controlling gene-gene interactions might never be possible. But this observation is not so straightforwardly true. Indeed, a large GRN means tens or hundreds of parameters to be tuned. They could just be flexible enough as to carry out a desired task with several different sets of parameters, or with parameters within certain ranges, thus dismissing the need for fine-tuning. ANNs implement computations in a distributed and emergent manner, usually avoiding that very specific pieces are responsible of very precise parts of the calculi. This makes ANNs both flexible and robust against stochastic events, and it is not strange that different ANNs work in a similar and stable way while having very different internal settings. These features are very interesting for synthetic circuits design.

It might not only be the case that fine-tuning is not required. Cutting-edge experimental techniques could allow us to treat whole genomes as black boxes upon which accelerated evolution aided by artificial selection could implement training algorithms similar in essence to a Hebbian learning. In such a case we would not need to know any actual numerical values for parameters that we would need to write down into a genome. Rather, the evolving system could self-organize to implement any function that we wished, as long as it would have enough computational complexity.

As already outlined in section III, our algorithm implementation required some assumptions. For example, we have chosen to use the constant Hill coefficient \( n = 2 \), or to consider both \( \alpha_{ij} \) and \( \beta_{ij} \) as the weights between pairs of units: this may enclose some redundancy. We have also used the most basic learning algorithm possible, while nowadays several techniques exist that make the training of a network far more efficient, meaning better performance and reduced learning times. This very basic algorithm was already enough for the proof of concept that we intended. However, all this work should be revised some day if the design of actual GRNs were sought: we should find the most economic networks, thus needing some tuning; and those networks working in parameter ranges in which rewiring or pruning techniques would be a nice extension; those networks working in parameter ranges in which less fine-tuning is required would also be preferred. Besides, designing networks that are robust to noise or that somehow incorporate it as a computing feature would be desirable, since stochasticity pervades real regulatory systems. All these questions can be addressed numerically and analytically using some of the methods developed in this paper, or incorporating to them whatever extensions were needed. Finally, similar algorithms can be derived for recurrent and dynamical models of GRNs. This would hopefully reveal an even greater power of networks of genes as computational devices.

Acknowledgements

We would like to thank the members of the Complex Systems Lab. This work has been supported by grants from the Spanish MINECO, a European Research Council Advanced Grant, the Botín Foundation and by the Santa Fe Institute.

References

[Alon 2006] Alon U (2006) An Introduction to Systems Biology: Design Principles of Biological Circuits. Taylor & Francis.
[Alon 2007] Alon U (2007) Network motifs: theory and experimental approaches. Nature 8:450-61
[Amos 2004] Amos M (2004) Cellular Computing. Oxford University Press
[Amos 2008] Amos M (2008) Genesis Machines: The New Science of Biocomputing. MIT Press, Cambridge MA
[Auslander et al. 2012] Ausländler S, Ausländler D, Müller M, Wieland M, and Fussenegger M (2012) Programmable single-cell mammalian biocomputers. Nature 487(7405):123-127
[Bashor et al. 2010] Bashor CJ, Horwitz AA, Peisajovich SG, and Lim WA (2010) Rewiring cells: synthetic biology as a tool to interrogate the organizational principles of living systems. Annu Rev Biophys 39:515-537
[Benenson 2012] Benenson Y (2012) Biomolecular computing systems: principles, progress and potential. Nature Rev Genet 13:455-468
[Bishop 2006] Bishop CM (2006) Pattern Recognition and Machine Learning. Springer-Verlag, New York
[Bratsun et al. 2005] Bratsun D, Volfson D, Tsimring LS, and Hasty J (2005) Delay-induced stochastic oscillations in gene regulation. Proc Natl Ac Sci 102(41):14593-14598
[Bray 1990] Bray D (1990) Intracellular Signaling as a Parallel Distributed Process. J theor Biol 143:215-231
[Bray 1995] Bray D (1995) Protein molecules as computational elements in living cells. Nature 376:307-312
[Brenner 2012] Brenner S (2012) Life’s code script. Nature 482:461
[Dayan and Abbott 2005] Dayan P and Abbott LE (2005) Theoretical Neuroscience. MIT Press, Cambridge MA
[Gardner et al. 2000] Gardner TS, Cantor CR, and Collins JJ (2000) Construction of a genetic toggle switch in Escherichia coli. Nature 403:339-342
[Goutelle 2008] Goutelle S, Maurin M, Rougier F, Barbaut X, Bourguignon L, Ducher M, and Maire P (2008) The Hill equation: a review of its capabilities in pharmacological modelling. Fund Clin Pharmacol 22:633-636
[Hasty et al. 2001] Hasty J, Isaacs F, Dolnik M, McMillen D, and Collins JJ (2001) Designer gene networks: Towards fundamental cellular control. Chaos 11(1):207-220
[Hill 1910] Hill AV (1910) The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J Physiol 40:iv-vii
[Hoffman-Sommer et al. 2012] Hoffman-Sommer M, Supady A, and Klipp E (2012) Cell-to-cell communication circuits.
quantitative analysis of synthetic logic gates. Front Physio
3:287

Jacob and Monod 1961] Jacob F and Monod J (1961) Ge-
netic regulatory mechanisms in the synthesis of proteins.
J Mol Biol 3:318-356

Kauffman 1993] Kauffman SA (1993) Origins of Order. Ox-
ford University Press

Kawczyński and Legawiec 2001] Kawczyński AL, Legawiec
B (2001) Two-dimensional model of a reaction-diffusion
system as a typewriter. Phys Rev E 54:056202-1

Kitano 2001] Kitano H (2001) oudations of Systems Biol-
ygy. MIT Press, Cambridge MA

Lim 2010] Lim WA (2010) Designing customized cell sig-
nalling circuits. Nat Rev Mol Cell Bio 11:393-403

Macía et al. 2009a] Macía J, Widdler S, and Solé RV (2009)
Specialized or flexible feed-forward loop motifs: a ques-
tion of topology. BMC Syst Biol 3:84

Macía et al. 2009b] Macía J, Widdler S, and Solé RV (2009)
Why are cellular switches Boolean? General conditions for
multistable genetic circuits. J Theor Biol 261(1):126-135

Macía et al. 2012] Macía J, Posas F, and Solé RV (2012) Dis-
tributed computation: the new wave of synthetic biology
devices. Trends Biotechnol 30(6):342-349

Maass et al. 2002] Maass W, Natschlaeger T and Markream
H (2002) Real-time computing without stable states: A
new framework for neural computation based on pertur-
bations. Neural Comput 14(11):2531-2560

Mattiausi and Floreano 2007] Mattiausi C and Floreano D
(2007) Analog genetic encoding for the evolution of cir-
cuits and networks. IEEE T Evolut Comput 11:596-607

McCulloch and Pitts 1943] McCulloch WS and Pitts WH
(1943) A logical calculus of the ideas immanent in ner-
vous activity. B Math Biophys 5:115-133

Mondragón et al. 2011] Mondragón-Palomino O, Danino T,
Slonimkov J, Tsirring L, and Hasty J (2011) Entrainment
of a Population of Synthetic Genetic Oscillators. Science
333:1315-1319

Nurse 2008] Nurse P (2008) Life, logic and information. Na-
ture 454:424-426

Olazaran 1989] Olazaran M (1989) A Sociological Study of
the Official History of the Perceptrons Controversy. Soc
Stud Sci 26(3):611-659

[Regot et al. 2010] Regot S, Macía J, Conde N, Furukawa K,
Kjellén J, Peeters T, Hohmann S, de Nadal E, Posas F,
and Solé R (2010) Distributed biological computation with
multicellular engineered networks. Nature 469:207-211

Rodriguez-Caso et al. 2005] Rodriguez-Caso C, Medina MA,
Solé RV (2005) Topology, tinkering and evolution of the
human transcription factor network. FEBS J. 272: 6423-
6434.

Rothemund et al. 2004] Rothemund PWK, Papadakis N,
Winfree E (2004) Algorithmic Self-Assembly of DNA Sier-
pinski Triangles. PLoS Biol 2(12):e424

Rumelhart et al. 1986] Rumelhart DE, Geoffrey EH, and
Williams RJ (1986) Learning representations by back-
propagating errors.

Saez-Rodriguez et al. 2009] Saez-Rodriguez J, Alexopoulos
LG, Epperlein J, Samaga R, Laubenfenger DA, Klamt S,
and Sorger PK (2009) Discrete logic modelling as a means
to link protein signalling networks with functional analysis
of mammalian signal transduction. Mol Syst Biol 5:331

Santillán 2008] Santillán M (2008) On the Use of Hill Func-
tions in Mathematical Models of Gene Regulatory Net-
works. Mat Model Nat Phenom 3(2):85-97

Sirbu et al. 2011 and references therein] Sirbu A, Ruskin
HJ, and Crane M (2012) Stages of Gene Regulatory Net-
work Inference: the Evolutionary Algorithm Role

Solé and Macía 2013] Solé RV and Macía J (2013) Expanding
the landscape of biological computation with syn-
thetic multicellular consortia. Nat Comp 10.1007/s11047-
013-9380-y

Tabor et al. 2009] Tabor JJ, Salis H, Simpson ZB, Chevalier
AA, Levskaya A, Marcotte EM, Voigt CA, and Ellington
AD (2009) A Synthetic Genetic Edge Detection Program.
Cell 137(7):1272-1281

Wang et al. 2009] Wang HH, Isaacs FJ, Carr PA, Sun ZZ,
Xu G, Forest CR, and Church GM (2009) Programming
cells by multiplex genome engineering and accelerated evo-
lution. Nature 460:894-899

Widder et al. 2012] Widder S, Solé R, and Macía J (2012)
Evolvability of feed-forward loop architecture biases its
abundance in transcription networks. BMC Syst Biol 6:7
Nature 323:533-536