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The Impact of Self-Loops on Boolean Networks Attractor Landscape and Implications for Cell Differentiation Modelling

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The impact of self-loops on Boolean networks attractor landscape and implications for cell differentiation modelling

Sara Montagna, Michele Braccini, and Andrea Roli

Abstract—Boolean networks are a notable model of gene regulatory networks and, particularly, prominent theories discuss how they can capture cellular differentiation processes. One frequent motif in gene regulatory networks, especially in those circuits involved in cell differentiation, is autoregulation. In spite of this, the impact of autoregulation on Boolean network attractor landscape has not yet been extensively discussed in literature. In this paper we propose to model autoregulation as self-loops, and analyse how the number of attractors and their robustness may change once they are introduced in a well-known and widely used Boolean networks model, namely random Boolean networks. Results show that self-loops provide an evolutionary advantage in dynamic mechanisms of cells, by increasing both number and maximal robustness of attractors. These results provide evidence to the hypothesis that autoregulation is a straightforward functional component to consolidate cell dynamics, mainly in differentiation processes.

Index Terms—Genetic regulatory networks model; Boolean networks; Self-Loops; Cell Differentiation

1 INTRODUCTION

Gene regulatory networks (GRNs) are usually characterised by feedback loops that enable regular oscillations, such as in the case of circadian and cardiac rhythms [1], and provide effective mechanisms for stabilising network dynamics [2]. A prominent context in which loops play a crucial role is cell differentiation, where these mechanisms enable cells to regulate gene expression. In this paper, we are interested in investigating the specific kind of feedback represented by an *autoregulation* not mediated by other genes. For the sake of clarity, in the following we will refer to this very kind of feedback as *direct autoregulation* [3].

Among the different models for GRNs [4], in this paper we focus on Boolean models [5], [6], [7], whose main assumptions are that genes are either active or inactive and that their interactions are modelled by Boolean functions. In spite of these simplifying hypotheses, Boolean models have been proven to successfully capture relevant phenomena in cell biology [8], [9], [10]. Surprisingly, the generic properties of Boolean networks exhibiting direct autoregulation have not yet been investigated. In these models, direct autoregulation can be easily introduced in the form of *self-loops*—directed arcs that exit from a node and are also input for the same node—that denote a functional dependence of a variable from itself. In a previous preliminary work [11], we have found that the impact of self-loops in random Boolean networks is to increase the average number of attractors and reduce their average robustness. This last result is not in agreement with biological networks, which are undoubtedly robust and yet contain self-loops.

In this work we shed light on this conundrum by

investigating in more depth the impact of self-loops in Boolean networks on both the number of attractors and their robustness. We first show that the number of nodes with a self-loop is indeed positively correlated with the average number of attractors and we provide a formal model for this relation. Subsequently, we show that, if we restrict statistics to networks with the same number of attractors, the maximal robustness of the attractors increases with the fraction of nodes with a self-loop. In other words, the advantage of self-loops is still observable in Boolean models but by comparing attractor robustness *ceteris paribus*. In addition, our results show that the variability of attractor robustness tends to increase with the fraction of nodes with self-loops. This outcome suggests that direct autoregulation may provide an advantage in the evolution of the basic dynamic mechanisms of cells.

These results provide further support to the use of Boolean networks for modelling cell dynamics (e.g. differentiation processes) and suggest that self-loops have to be taken into account in the *ensemble approach* [12], which aims at identifying generic properties so as to match some statistical features of the target biological systems.

August 9, 2019

2 BACKGROUND AND MOTIVATION

Boolean networks (BNs) have been introduced by Kauffman [5], [13] as GRN models and have been shown to suitably capture important phenomena in biology [14], [15], [16], [17], [18], [19], [20], [21]. A Boolean network is a discrete-time discrete-state dynamical system whose state is a n -tuple in $\{0, 1\}^n$, (x_1, \dots, x_n) . The state is updated according to the composition of n Boolean functions $f_i(x_{i_1}, \dots, x_{i_{k_i}})$, where k_i is the number of inputs of node i , which is associated to Boolean variable x_i . Each function f_i governs the update of variable x_i and depends upon

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the value of variables $x_{i_1}, \dots, x_{i_{k_i}}$. A prominent BN model is that of random BNs (RBNs), characterised by a random topology (typically every node has exactly k inputs) and random Boolean functions defined by setting the probability to assign value 1 to each entry with probability b , called *bias*. In its original—and most studied—formulation the k incoming nodes are chosen randomly among the other $n - 1$ nodes, without repetition and avoiding self-loops. In this work we will refer to the synchronous and deterministic BN update, whereby nodes update their state in parallel and Boolean functions are deterministic. Under this update scheme, each network state has only one successor and the asymptotic states of the system are cyclic attractors or fixed points.

Being the asymptotic states of the system, attractors play a prominent role in BNs [5], [22]. In particular, a recent dynamical systems view of cell differentiation has been proposed [15], [16], [18], [23] in which attractors—or subsets of attractors—represent cell types. Accordingly, transitions between attractors epitomise cell differentiation stages that bring changes in the pattern of active/inactive genes. In this view, for a network to be a viable model for cell differentiation, one would require to have a suitable number of attractors characterised by varying degrees of robustness, so as to be able to reproduce the transitions between cell types.

In [11] we presented a preliminary study that analyses whether adding self-loops in RBNs affects attractor number and type and, possibly, their robustness. We observed that the number of attractors is higher in networks with self-loops and grows quasi-exponentially with the number of self-loops. At the same time, attractor robustness tends to be smaller than in RBNs without self-loops.

These results are not completely coherent with the role self-loops have in biological systems. Indeed, autoregulatory circuits—biological components that are (in)directly influenced by their very product—are pervasive in biological organisms and they are actively involved in conferring mutational, environmental, recombinational, or behavioural robustness. The effects of these circuits manifest themselves as emergent properties on multiple scales, in time (development/evolution), in space (populations) and on different levels of the biological organization (from molecular up to entire organisms). Buffering of noise and incomplete penetrance [24], autocatalysis, homeostasis and buffering gene dosage [25], genetic switches—like *Sxl* gene in sex determination of *Drosophila* [26] and *Cl protein* in lytic or lysogenic phase control in bacteriophage lambda [3]—and chromatin mediated autoregulation [27] are just some of the most prominent examples of the observable effects of positive or negative, direct or indirect autoregulatory circuits.

Autoregulation patterns assume particular relevance in transcriptional regulation. Indeed, in the works by Alon and colleagues [2], [28], [29], autoregulatory circuits have been identified in transcription networks as network motifs, i.e. recurring building-block patterns found in complex networks. Just to mention an example of their amount in a real organism, *E. coli* presents 40 transcription factors that regulate the transcription of their own genes, out of a total

of 420 transcription factor encoding genes.¹

Particularly noteworthy to the purpose of this paper are the functions that positive autoregulations carry out in differentiation, and therefore in development. According to [28], positive (negative) autoregulation occurs when a transcription factor enhances (represses) its own rate of production. It is the memory capacity typical of positive circuits made by maintaining gene expression, and so acting as genetic switches, that makes them important in biological development. Therefore, autoregulation of important developmental regulatory proteins can *lock-in* their expression and so induce the maintaining of attained cell fates or developmental states [3]. Thomas in his works [25], [26], [30] remarks the key role of autoregulation in the context of cell differentiation firstly by demonstrating that a positive loop is necessary for multistationarity and subsequently, following the hypothesis of Delbrück, that differentiation represents the biological aspect of the latter. In addition, Alon [2] addresses a mathematical systematic study of the mechanisms characterising autoregulation generic dynamical properties: negative autoregulation speeds-up transcription response time, whilst positive ones slow down the transcription factors response time and are able to create bi-stability. Huang et al. [31] study the interactions between two key transcription factors in blood differentiation, namely GATA1 and PU.1, to understanding the discrete cell fate decisions that multipotent cells undergo during development. These two transcription factors promote the erythroid or myelomonocytic lineage respectively. In a dynamical systems vision of cell differentiation, the authors formulate a minimal mathematical model of the functional interactions of the two above-mentioned transcription factors that in a qualitatively way reproduce—in the GATA1 and PU.1 plane—the observed experimental genome-wide trajectories of the transcriptome during differentiation. In addition to mutual inhibition, auto-stimulation of GATA1 and PU.1 turned out to be fundamental to give rise to the metastable state characterized by the promiscuous expression of both transcription factors and representing the progenitor cells.

In the light of the previous—non-exhaustive—list of biological and modelling examples in which autoregulations play relevant roles in the organism functions, especially in transcription networks, we cannot ignore their roles also in Boolean models of GRN. In fact, Boolean reconstructed models of GRNs, obtained by making use of consolidated biological knowledge of transcription factors interactions or following *ad-hoc* procedures for synthesizing models able to reproduce observed data, are characterised by autoregulations. As an example we cite the reconstructed Boolean network representing the core endogenous network of early myeloid cell-fate determination [32] that presents nearly 10% of nodes with self-loops. In [33] the authors have reconstructed a BN model of the control mechanisms that drive the epithelial-to-mesenchymal transition (EMT). In that minimal GRN model autoregulations are necessary to create the required stable attractor states and, in particular, the hybrid cell state that presents in the gene expression profile both the epithelial and mesenchymal features. Moreover,

1. according to [2]

by analysing the database (<https://cellcollective.org/#>) of Boolean-rule based computational models of large-scale biological networks available at the Cell Collective website [34], we have ascertained that the 54% of networks has self-loops. Nevertheless, a systematic study aimed at identifying **generic properties** and qualitatively characterising the impact of nodes with self-loops in BNs is missing. Indeed, modelling and analysing specific genetic circuits is a valuable and necessary step to pursue an ever-increasing understanding of the mechanisms that underlie biological organisms, but it suffers from limitations because these circuits are not actually functionally independent [31]. Therefore, in order to frame up their effects in the dynamics of the complex networks of which they belong, it is necessary to address also this study in terms of generic properties. Our work is in the track of the long-term research aiming at constructing a **synthetic network biology** theory since self-loop may represent a possible **functional bricks**.

3 METHODS

The experiments we performed concern RBNs in which the main factor we control is the fraction of nodes with a self-loop. Starting from a RBN with n nodes denoted by integer values $V = \{1, 2, \dots, n\}$, k inputs per node and bias b , a self-loop can be introduced in a node either by rewiring an input or by adding a new one. The function of nodes with self-loops may be arbitrarily altered or left random. Two are the main reasons for investigating the impact of autoregulation as self-loops in random Boolean models: the first is that BNs are among the most used GRN models and a wealth of results on RBNs is already available, therefore we can compare our findings against an established and well known literature. The second reason is that if self-loops make RBNs somehow more adapt to model differentiation processes, then they should be taken into account in artificial evolution experiments and RBNs with self-loops may provide a promising initial condition for such studies.

We performed the experiments according to the following experimental setting. Initial RBNs were created with $k = 2$ and function bias b equal to 0.5—the value of these parameters grounds on biological plausibility [13], [35], [36]. These networks have been modified by selecting at random n_s nodes in which rewiring or adding a link in self-loop. For brevity, let's denote by $V_s \subseteq V$ the nodes with a self-loop. When self-loops are introduced by rewiring, all nodes in V have exactly k inputs; conversely, when self-loops are added, the distribution of node in-degree changes as n_s nodes out of n have $k + 1$ inputs. We decided to test both variants so to have a wider picture of the effects of self-loops inserted in RBNs. Besides adding self-loops, a decision has to be taken concerning the functions of nodes in V_s : since we started from RBNs, the function may still be random (with bias kept to b also in the case $k + 1$) or it can be set to a specific Boolean function. This latter case is the most relevant for GRN modelling, as self-loops found in biological cells have usually a canalising role [37] instead of playing any function. As originally introduced by Kauffman [5], a canalising function is a Boolean function in which there exists an input value that fully determines the output value, regardless of the values of other inputs.

Table 1: Topological Configuration of Networks

Configuration	Description
CONST-OR	removed an incoming link and replaced the input with a self-loop, and changed the node Boolean function into an OR
AUGM-OR	added a self-loop and changed the node Boolean function into an OR between the node value and the previous function
CONST-RND	removed an incoming link and replaced the input with a self-loop, without changing the node Boolean function
AUGM-RND	added a self-loop and extended the truth table randomly (with the same bias used for generating the original RBN)

We then chose to test also the case in which the Boolean function of nodes in V_s is a either logical OR between the value of the node itself and the other input (k constant case) or the OR between node value and the random function initially set, for the $k + 1$ case. Formally, let i be a node in V_s and f_i the original function; then, the new Boolean function \hat{f}_i is defined as follows:

- case k constant: $\hat{f}_i = x_i \vee x_j$, where j is the other input of i ;
- case $k + 1$: $\hat{f}_i = x_i \vee f_i$.

Note that in BN models there is actually no semantics associated to 0 and 1. However, since for the sake of simplicity we chose one specific canalising function—the logical OR—this implies that if 1 is associated with the active state of the node, OR acts as a canalising activating function—and, clearly, OR with self-loops means self-activation. Anyway, if we observe a specific effect of the OR function, it just means that this effect can be achieved by any canalising function of this kind. Our main interest is indeed on this canalising functional role, whilst the case with random functions is kept just for comparison. In Table 1 we sum up the set of BN variants used in the experiments we performed.

Attractors in BNs are unstable with respect to perturbations (i.e. temporary node value flips), therefore after a node flip the trajectory either returns to the same attractor or it reaches another one [38], [39]. Attractor transition probabilities are computed by exerting perturbations to each attractor and the probability of returning to an attractor after a perturbation is taken as an estimation of *attractor robustness*. According to [40], robustness is a broader concept than stability—which is a well-defined mathematical notion in dynamical systems—as it is related to *feature persistence* under a wider spectrum of perturbations of different nature. The notion of attractor robustness used in this paper, which is related to the concept of *robust adaptation* defined by Kitano in [41], is not limited to determine single attractor's stability since it provides a quantitative measure, i.e. the probability of returning to the same attractor.

This metric is clearly a function of the kind of perturbation exerted. In this work we suppose that only one node at a time can be perturbed and that only states belonging to an attractor can be subject to such a perturbation. This approach is common in dynamical systems, in which stability is indeed evaluated in stationary states. Moreover, the single flip hypothesis is based on the assumption that

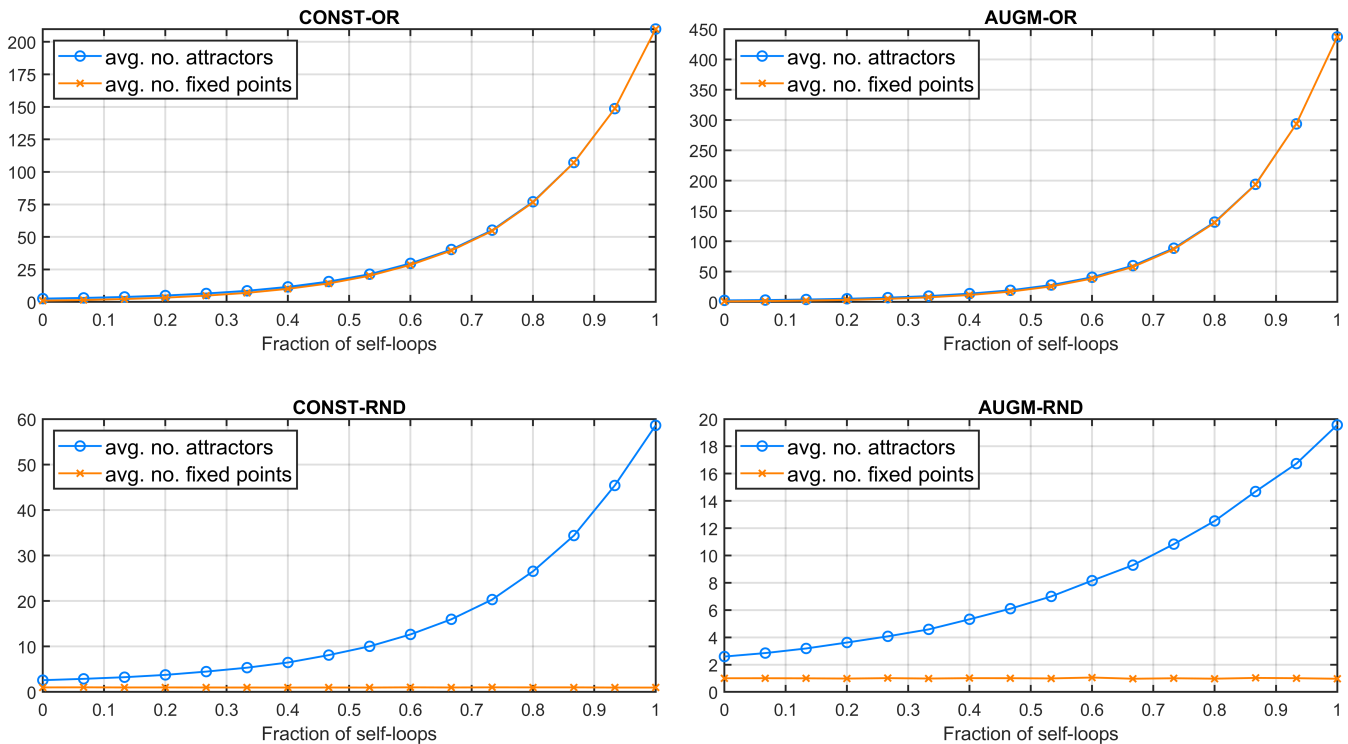


Figure 1: Average number of attractors in $2 * 10^4$ RBNs with 15 nodes as a fraction of self-loop varying from 0 to 1. Results are shown for each of the four configurations.

perturbations are not frequent with respect to network updates, so the probability of affecting more than one node at a time is negligible; the same consideration holds for perturbations occurring during transients, which typically last a tiny fraction of time with respect to attractors along BN trajectories. This assumption is reasonable in particular when BNs are used to model cell dynamics [14], [17], [39].

In practice, we apply a logic negation to each node of each state of each attractor in turn and we check in which attractor the dynamics relaxes. The probability of a transition between attractor A and attractor B is computed by taking the frequency of transitions between A and B among all the possible node flips along attractor A .² As shown in [42], the results obtained in this setting are equivalent to stochastic simulation of perturbed BNs. This procedure produces a probability transition matrix, that we name the *Attractor Transition Matrix* (ATM), from [43]. The diagonal of the ATM accounts for attractor robustness, as diagonal values represent the probability of returning to the same attractor after a perturbation. To get a more accurate evaluation of attractor robustness, in this work we focus on minimum and maximum values of the ATM diagonal, rather than one single statistics such as the average that might obfuscate the actual features of the distribution. To this purpose we define the two following variables:

$$\delta_{min} = \min \text{diag}(ATM) \quad (1)$$

2. For large size networks one has to resort to sampling, instead of enumerating all the possibilities.

$$\delta_{max} = \max \text{diag}(ATM) \quad (2)$$

4 RESULTS

In this section, we will first show the results on the number of attractors. Subsequently, we will analyse statistics on attractor robustness.

We run experiments for each of the four configurations described in Table 1. In this work, we aim at providing a detailed picture of the impact of self-loops on BNs and so we choose completeness over statistics in the large. Therefore, the number of nodes n is set to 15 so as to be able to perform an exact computation of the ATM for every possible number of nodes with self-loops $n_s \in \{0, 1, \dots, n\}$. The outcome of our study can be anyway generalised to large size networks. However, small and medium-size networks are often used to model the relations among a limited number of genes, related to a specific function (e.g., the hematopoietic cell differentiation). RBNs are generated with $k = 2$ and bias $b = 0.5$. For each value of n_s and for each variant, we took statistics across $2 * 10^4$ randomly sampled RBNs.

4.1 Average number of attractors as a function of self-loops

In this section we show how the average number of attractors is affected by self-loops. Figure 1 is composed of four plots, each referring to one of the four configurations in Table 1. In each graph, the average number of attractors

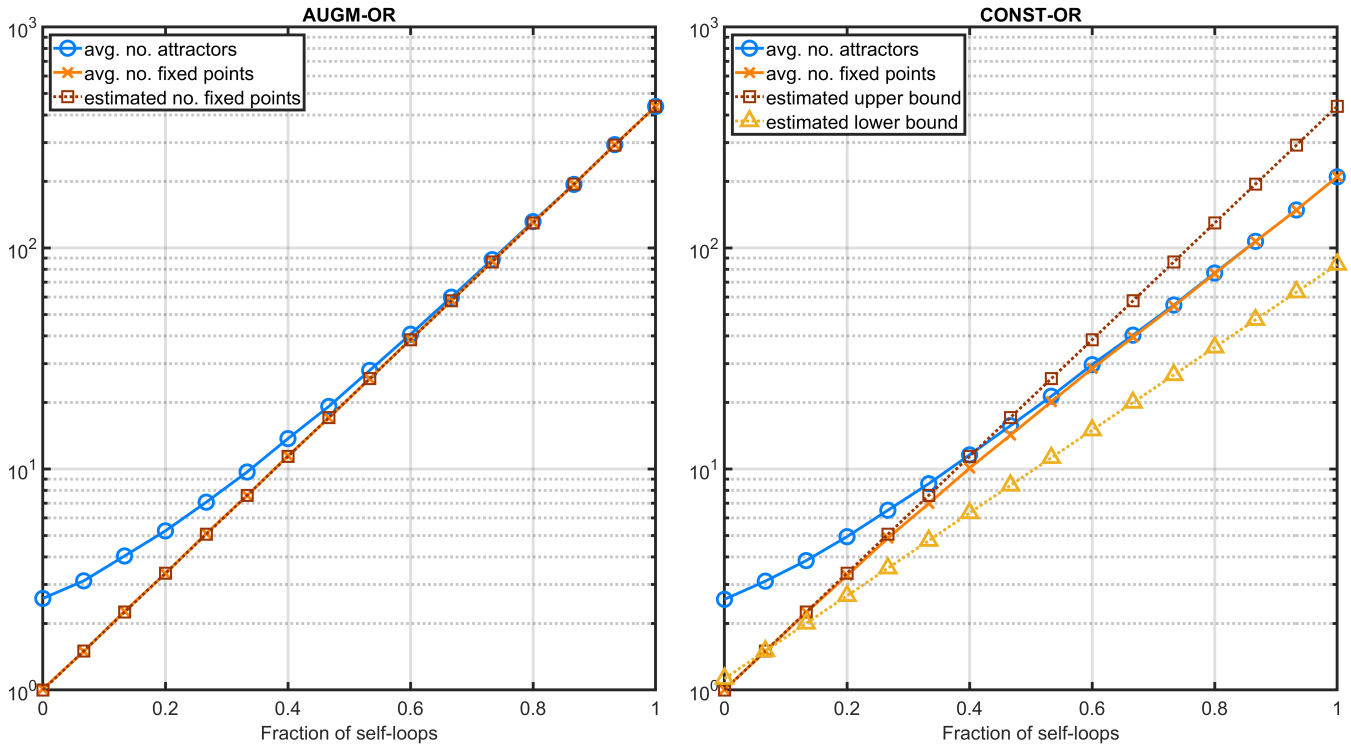


Figure 2: Comparison between the mathematical model and experimental results. Self-loop in OR inserted by adding an input (left), and by rewiring one input (right). Logarithmic scale for the y axis.

is plotted as a function of the fraction of nodes with self-loops, along with the average number of fixed points. The outcome of these experiments confirms the results presented in our previous work [11]: the average number of attractors increases with n_s/n . Curves follow the same trend in the four plots and are well approximated by an exponential function. However, we can observe two main differences between OR (canalising functions) and RND functions:

- 1) The number of attractors increases much more in networks with self-loops in OR than in those with RND functions. The difference is roughly of an order of magnitude: we can observe that with OR they vary from an average value of 2.59 in all the networks without self-loops until averages of 209.8 (CONST-OR), 436.9 (AUGM-OR), 58.6 (CONST-RND) and 19.6 (AUGM-RND) in networks with 15 self-loops.
- 2) For CONST-OR and AUGM-OR, almost all attractors are fixed points even at low values of n_s/n ; conversely, in the RND cases, is the average number of cyclic attractors that grows with n_s/n .

4.1.1 An analytical model

To generalising the previous results and being able to make predictions for any value of n , n_s and bias b , we complemented this analysis with a theoretical estimation of the average number of attractors as a function of these parameters. The model we provide in this section is related to the OR cases, as they are more significant for biological cell modelling than the RND ones. As previously observed,

in the OR cases even for few nodes with self-loops almost all attractors are fixed points. Therefore, as we want to have a generalisation for any n , we estimate the number of attractors in terms of number of fixed points. For ease of the proof, we first focus on AUGM-OR and we subsequently modify the model for the CONST-OR case. Our goal is then to estimate the probability that a randomly chosen state $s = (x_1, x_2, \dots, x_n)$ is a fixed point in the case in which self-loops are added to nodes. In the following, we use $f_i(\cdot)$ to denote the application of the function of node i to its inputs values.

Hence we want to estimate:

$$p^*(s) = P\{s \text{ is a fixed point} \mid s \text{ is randomly chosen}\} \quad (3)$$

s is a fixed point iff $s = F(s)$, i.e. $(x_1, x_2, \dots, x_n) = (f_1(\cdot), f_2(\cdot), \dots, f_n(\cdot))$.

Let us focus on node i with two external inputs corresponding to a Boolean function with bias b and a self-loop in OR and estimate the probability $p_i^* = P\{x_i = f_i(\cdot)\}$. We have two cases: (a) $x_i = 1$ and (b) $x_i = 0$.

$$a) P\{x_i = 1 \wedge f_i(\cdot) = 1\} = P\{f_i(\cdot) = 1 \mid x_i = 1\} P\{x_i = 1\} = 1 \cdot q = q, \text{ where } q \text{ is the probability of assigning 1 to value } x_i.$$

$$b) P\{x_i = 0 \wedge f_i(\cdot) = 0\} = P\{f_i(\cdot) = 0 \mid x_i = 0\} P\{x_i = 0\} = (1 - b)(1 - q), \text{ because } b \text{ is the probability that—on average—} f(\cdot) = 1.$$

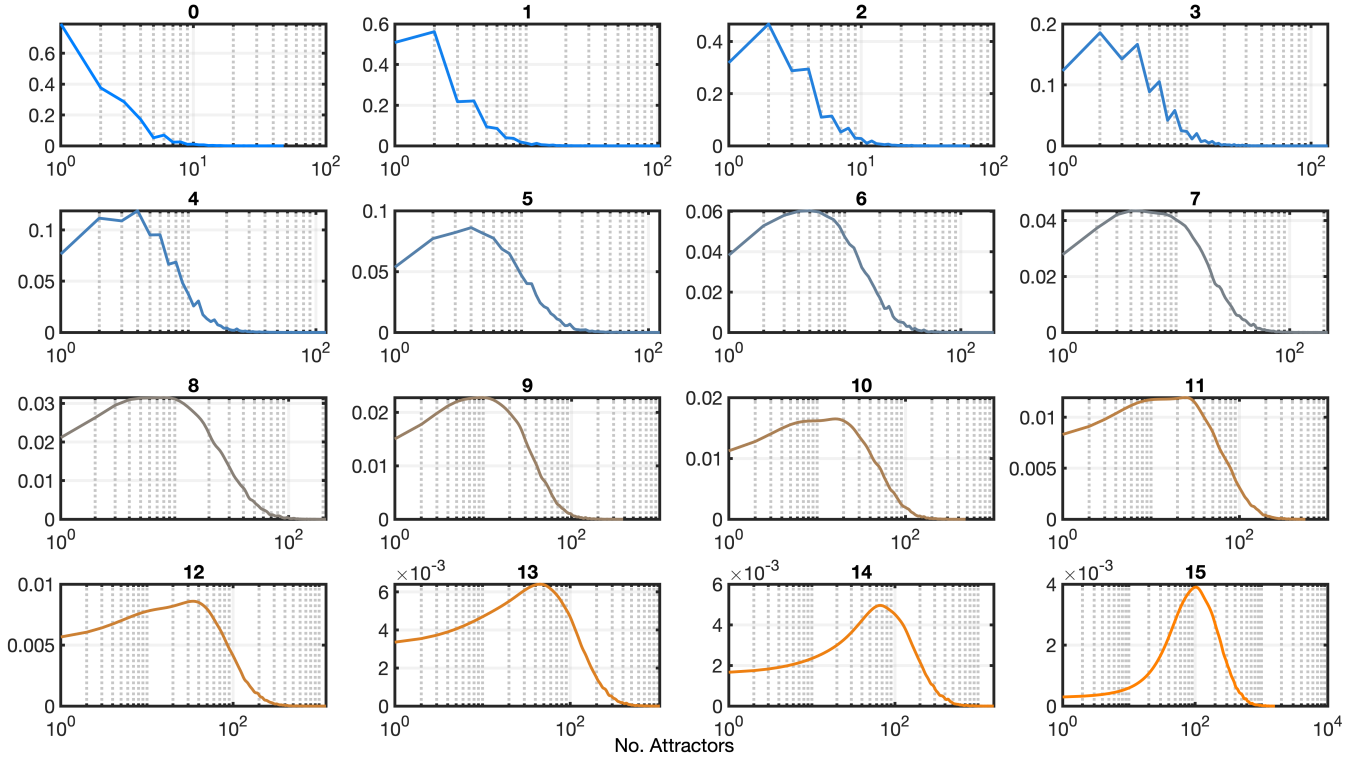


Figure 3: Density function of the number of attractors for the CONST-OR configuration. It has been computed from experimental data on 2×10^4 runs with same n_s . The sixteen plots show how it changes with $n_s \in \{0, 1, \dots, 15\}$ (the number of self-loops n_s is reported in bold letters in the title of each plot). Logarithmic scale for the x axis.

Hence, $p^*(x_i) = q + (1 - b)(1 - q)$. We suppose that initial states are randomly chosen, thus $q = \frac{1}{2}$. Moreover, in our experiments we have $b = \frac{1}{2}$, therefore $p^*(x_i) = \frac{3}{4}$. For a node without self-loops we apply an analogous argument and obtain $\bar{p}^*(x_i) = bq + (1 - b)(1 - q)$; in our experiments $\bar{p}^*(x_i) = \frac{1}{2}$. Finally, we can derive a formula for the probability of a fixed point of a network with n nodes and n_s nodes with a self-loop added in OR under the hypothesis that all p_i^* are independent:

$$p^*(s) = [q + (1 - b)(1 - q)]^{n_s} [bq + (1 - b)(1 - q)]^{(n - n_s)} \quad (4)$$

For $b = \frac{1}{2} = q$ we have $p^*(s) = (\frac{3}{4})^{n_s} (\frac{1}{2})^{n - n_s}$.

The comparison between the theoretical value of fixed points and its experimental estimation—based on the statistics on BNs we have performed from simulations of RBNs—is shown in Figure 2 (left). The model predicts with high precision the number of fixed points, which is a good estimation of the overall number of attractors. The model slightly overestimates the number of fixed points because we suppose that nodes are independent—in fact, the functional dependence among nodes might rule out some configurations that can, in principle, be fixed points. However, this discrepancy is negligible in this case.

Following a similar reasoning we can derive an analogous formula for the CONST-OR case, in which self-loops are inserted by rewiring an incoming arc and substituting the Boolean function with an OR. In this case we have:

$$p^*(s) = [q + \frac{1}{2}(1 - q)]^{n_s} [bq + (1 - b)(1 - q)]^{(n - n_s)} \quad (5)$$

For $b = \frac{1}{2} = q$ we have $p^*(s) = (\frac{3}{4})^{n_s} \frac{1}{2}^{(n - n_s)}$.

The constant value $\frac{1}{2}$ in Equation 5 represents the probability that—on average—variable x_i with value 0 does not change its value after the application of the OR function.

The comparison between the theoretical value of fixed points and its experimental estimation is shown in Figure 2 (right, dotted line with squares). As we can observe, in this case the hypothesis of independence introduces an error, especially when the number of nodes with self-loop is high. For this case we should apply the chain rule for computing the conjunct probability that every node is constant. Let us denote by \bar{x}_i the event that x_i does not change its value after the update. Thus we have: $p^*(s) = P\{\bar{x}_1 \wedge \bar{x}_2 \wedge \dots \wedge \bar{x}_n\} = P\{\bar{x}_1\} P\{\bar{x}_2 | \bar{x}_1\} P\{\bar{x}_3 | \bar{x}_2 \wedge \bar{x}_1\} \dots P\{\bar{x}_n | \bar{x}_{n-1} \wedge \dots \wedge \bar{x}_1\}$.

The dependence among nodes can be simplified because in this topology the nodes with self-loop depend only on one other node as in a ring topology. Hence: $p^*(s) \approx P\{\bar{x}_1\} P\{\bar{x}_2 | \bar{x}_1\}^{n_s - 1} = \frac{3}{4} (\frac{2}{3})^{n_s - 1} (\frac{1}{2})^{n - n_s}$, as in the most constrained case x_2 depends on x_1 and, in this case, x_2 is constant in two out of the three cases in which x_1 is constant. In this way, we can provide also a lower bound on the number of fixed points, as depicted in Figure 2 (right).

To generalise analytical model results, Figures SM16 and SM17 of Supplementary Material report model predictions varying the bias for the analysed topological OR configurations (CONST-OR, AUGM-OR). We can observe that in the CONST-OR configuration the bias value does not impact the theoretical value of fixed points, while in the AUGM-OR

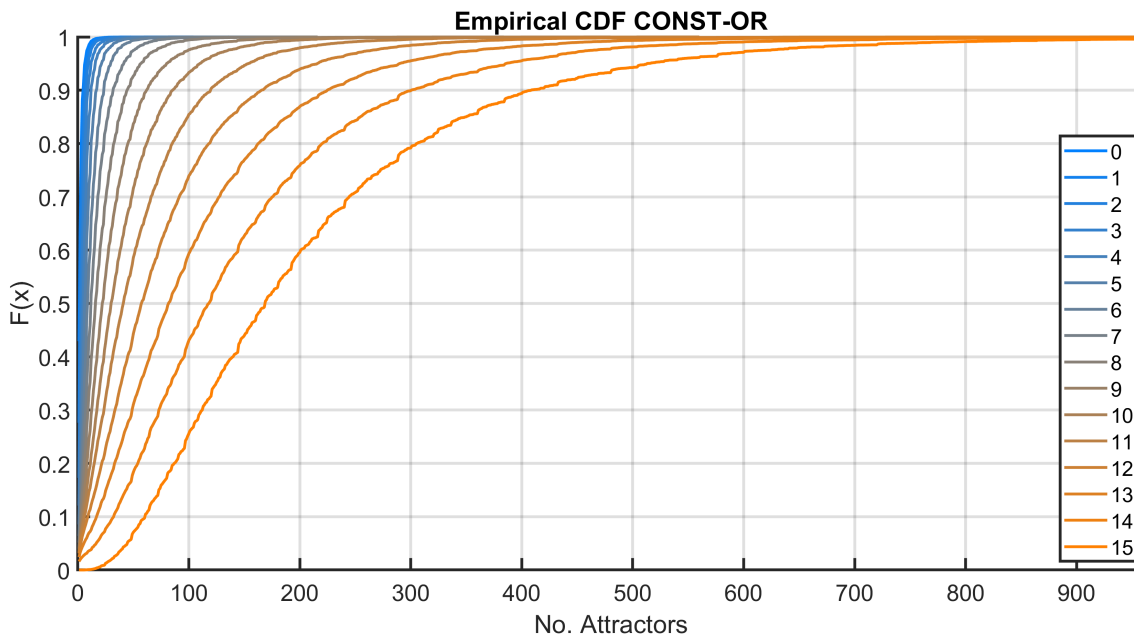


Figure 4: Empirical cumulative distribution function (ECDF) of the number of attractors for the CONST-OR configuration. It has been computed from experimental data on $2 * 10^4$ runs with same n_s , with $n_s \in \{0, 1, \dots, 15\}$ (see legend).

case analytical model predictions change with bias values.

As a final note, we observe that the model indeed capture an exponential relation between the average number of attractors and the fraction of nodes with self-loops, as empirically noted in Figure 1.

For the sake of completeness, we also introduce the analytical model for the estimation of the average number of fixed points for the RND cases. The model presented above can be reduced to the following formula $[bq + (1-b)(1-q)]^n$: since all nodes have random functions we can not make a distinction between nodes with a self-loop n_s and those without. Given that the average outcome of the Boolean functions in RBN ensembles of random functions follows the bias parameter, in Figure SM18 we report the theoretical estimations of the average number of fixed points varying bias values for the RND cases (CONST-RND, AUGM-RND). It is noteworthy that, for both the RND cases, the theoretical average number of fixed points is 1—regardless of the bias—and the experimental value reported in Figure 1 is perfectly in agreement with it.

4.2 Distribution of attractor number

To providing a more detailed picture of the overall attractor number trend we analysed the distribution of the number of attractors of the BNs across the $2 * 10^4$ networks with same configuration and number of self-loops. Moreover, this analysis is useful to study attractor robustness, which is influenced by the number of attractors, i.e. by the ATM size: in BNs a higher number of attractors is likely to correspond to a lower probability of returning to the same attractor after a perturbation, as on average the more the attractors, the

smaller their basin of attraction.³ For the sake of brevity, we discuss here the case of CONST-OR, and we refer to Supplementary Material for additional data and results on the other three configurations.

Figure 3 and Figure 4 show the density and the empirical cumulative distribution of the number of attractors, respectively, for each possible value of the number of self-loops in the network.

Density functions in plots of Figure 3 are computed with the algorithm implemented in `ksdensity` of Matlab (R2018a). The function returns a probability density estimate for the vector data containing the attractor number from our $2 * 10^4$ experimental results. The result is somehow surprising: for each network setting with at least one self-loop, the density is non-monotonic and a peak can be identified; in other terms, the probability of randomly sampling a network with a given number of attractors is not uniformly distributed, but rather most networks have a number of attractors close to the value corresponding to the maximal density. This peak varies with the number of nodes with self-loops. For instance, in networks without self-loops the value of the density function for a landscape with only one attractor is 0.79, while, once rewiring one node input with a self-loop, two attractors constitute the most common landscape and we have a value of 0.56. Peak value and position for the 16 experiments are summarised in Table 2. Peaks of density functions move right as self-loops are added to the network: the probability to have few attractors decreases while the probability to have a large number of attractors increases. Moreover, the peak gets lower while tails of density function get higher and longer. Generally

3. In fact, this is a rough reckon that holds on average, because the values in the ATM are computed by considering single perturbations occurring along attractor states, while the attractor basin is defined in terms of a fraction of the entire state space.

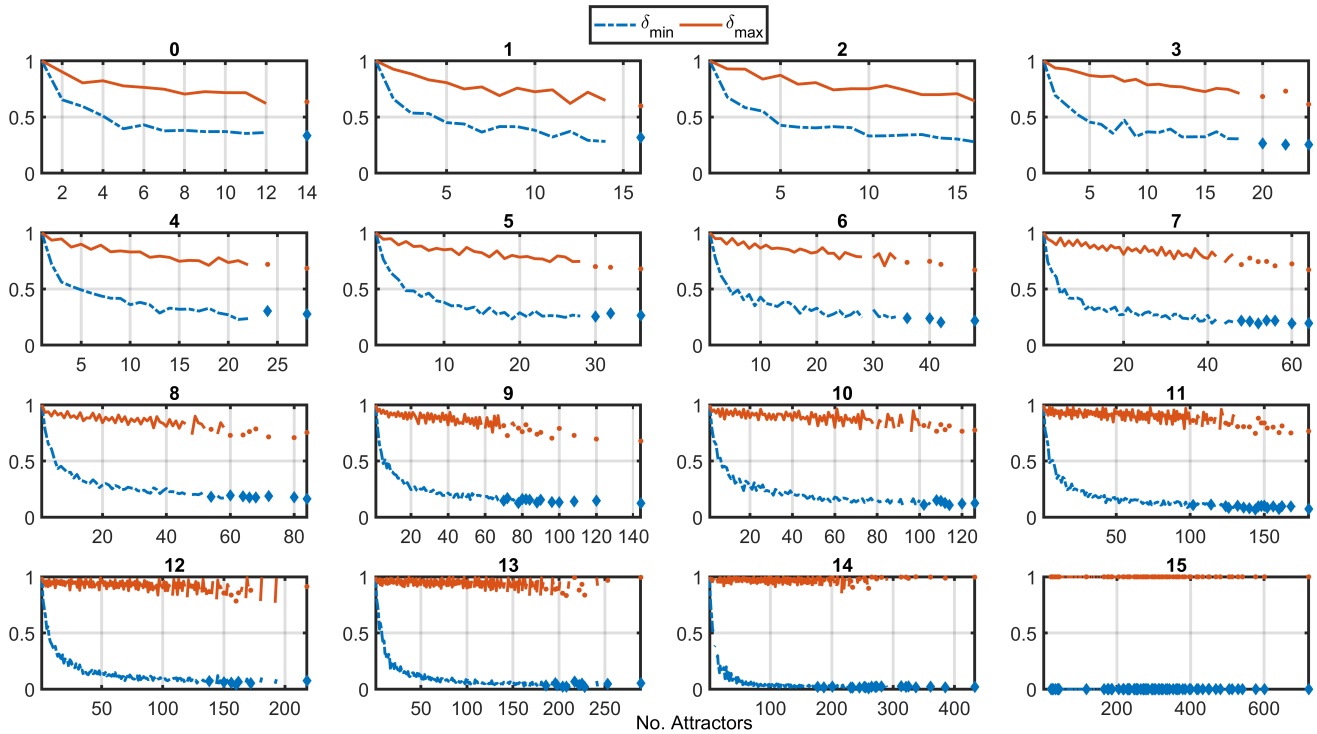


Figure 5: δ_{min} and δ_{max} as a function of the number of attractors m for the CONST-OR configuration. They have been computed from experimental data on $2 * 10^4$ runs with same n_s . The sixteen plots show how they change with $n_s \in \{0, 1, \dots, 15\}$ (the number of self-loops n_s is reported in bold letters in the title of each plot). x axis limits change with n_s from 1 to the largest number of attractors for which at least 30 sampled BNs have been found.

speaking the peak moves to the right while it drops down. Accordingly, we extracted the maximum number of attractors observed (A_{max}) as a function of the number of self-loops n_s (see Table 3).

A similar qualitative behaviour is obtained with the other three configurations (AUGM-OR, CONST-RND, AUGM-RND), has shown respectively in Figure SM1, Figure SM6 and Figure SM11 of Supplementary Material.

A different perspective is shown in Figure 4 where we plotted the empirical cumulative distribution function (ECDF) of each of the 16 different attractors number distributions related to the CONST-OR configuration presented in Figure 3 with $n_s \in \{0, 1, \dots, 15\}$. Figure 4 supports our previous analyses showing that 95% of networks are under the ECDF curve for attractor number gradually bigger. As extreme examples: if $n_s = 0$ then $ECDF \leq 0.95$ for $i \leq 6$, while if $n_s = 15$ then $ECDF \leq 0.95$ for $i \leq 512$.

A generalisation of this pattern can be attained by finding a fit between the ECDF of attractor number and a known discrete distribution. Indeed, for few self-loops the distribution is well fitted by a Poisson distribution. Unfortunately, this fit considerably degrades with increasing number of self-loops; in this latter case, we found a good match with a geometric distribution which tends to reproduce with more accuracy the tail of the attractor number distribution. Nevertheless, the geometric distribution completely misses the peak of the density. Even though continuous, the Weibull distribution [44] provides an overall good trade-off, being defined as a function of two parameters. This result would

Table 2: Density function max values and corresponding position, i.e. number of attractors.

n_s	density fun. max value	$x \equiv$ no. attractors
no self-loops	0.7904	1
1 self-loop	0.5619	2
2 self-loops	0.4673	2
3 self-loops	0.1857	2
4 self-loops	0.1187	4
5 self-loops	0.0861	4
6 self-loops	0.0603	5
7 self-loops	0.0435	4
8 self-loops	0.0315	8
9 self-loops	0.0228	10
10 self-loops	0.0165	16
11 self-loops	0.0119	25
12 self-loops	0.0086	34
13 self-loops	0.0064	45
14 self-loops	0.0049	67
15 self-loops	0.0039	102

suggest that the distribution of the number of attractors of the BNs is a mixture of exponential and Rayleigh distribution. A formal study of this issue is planned for future work.

4.3 Attractor robustness

The second relevant feature affected by the number of nodes with self-loops is attractor robustness. In our previous

Table 3: Max number of attractors as a function of self-loops n_s

n_s	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A_{max}	48	104	66	136	120	123	192	216	212	384	480	560	1200	1050	1545	1601

preliminary study [11] we observed that the median value of attractor robustness decreases with the number of self-loops. In fact, the distribution of these values is rather wide and a single statistical parameter might miss important features of the phenomenon. Moreover, as the distribution of the number of attractors of the BNs is not uniform, a fair comparison should be achieved by comparing robustness among BNs with the same number of attractors. Therefore, we look here at the minimum and maximum values of robustness— δ_{min} and δ_{max} —averaged across networks with the same number of attractors. In this way we have more balanced results across the 16 different settings, on top of which we can make a comparison and discussion: are self-loops affecting attractor robustness in networks with a different setting but same number of attractors? We computed averages of δ_{min} and δ_{max} on 30 networks randomly selected from the sampled pool of BNs with a given number of attractors; in case 30 networks are not available, statistics are not computed.⁴ We stop computing the averages at the largest number of attractors for which at least 30 sampled BNs have been found.

Figure 5 shows the trend of δ_{min} and δ_{max} as a function of the number of attractors across all 16 configurations of self-loops. We can observe that, in each plot, attractor robustness decreases with an increasing number of attractors: both δ_{min} and δ_{max} follow a quasi-monotonically decreasing function (with few local exceptions). This confirms the overall picture we got in our previous work. Results obtained for 15 self-loops has to be interpreted with a bit of ingenuity. In this case, $\delta_{min} = 0$ for all the networks and $\delta_{max} = 1$, independently of the number of attractors. The reason of this phenomenon is that in these peculiar networks in which every node regulates itself in OR, there are at least two fixed point attractors: $(0, 0, \dots, 0)$ and $(1, 1, \dots, 1)$. The first is unstable, as any node flip will switch to 1 at least one node which will keep this value forever; whilst the second is stable for every possible single flip, as any node perturbation $1 \rightarrow 0$ will be immediately reverted at the subsequent update step. In general, we can observe a tendency of δ_{min} to decrease, while δ_{max} increases with the number of self-loops, denoting that variability in attractor robustness increases. This trend is shown in Figure 6.

If we restrict the analysis to a limited number of attractors feasible for BNs with any number of self-loops and we consider δ_{max} at a given number of attractors m , we observe a notable fact: the maximum robustness grows with the number of self-loops (see Figure 7, right—the trend is shown for $1 \leq m \leq 20$). The picture emerging from the analysis of δ_{min} (Figure 7, left) is somewhat more complicated: the trend of minimum robustness is increasing for BNs with

4. Indeed, as for some topological configurations and ranges of n_s the computational cost for finding a RBN with a given number of attractors might be extremely high, we considered as statistical significant only averages computed across 30 samples, which is the minimal number suggested by a commonly applied rule of thumb [45].

few attractors and it decreases in networks with more than 12 attractors, while it does not significantly vary for an intermediate number of attractors.

Analogous results, even if less striking, hold for the AUGM-OR. Conversely, the RND cases do not show this behaviour. Figures summarising results for these settings can be found in Supplementary Material. Figure SM3 (AUGM-OR), Figure SM8 (CONST-RND) and Figure SM13 (AUGM-RND) show δ_{min} and δ_{max} as a function of the number of attractors m : the sixteen plots in each figure show how they change with $n_s \in \{0, 1, \dots, 15\}$. Figure SM4 (AUGM-OR), Figure SM9 (CONST-RND) and Figure SM14 (AUGM-RND) show δ_{min} and δ_{max} and ATM diagonal values as a function of the number of attractors. Results are shown for $n_s \in \{0, 4, 8, 12\}$. Figure SM5 (AUGM-OR), Figure SM10 (CONST-RND) and Figure SM15 (AUGM-RND) show δ_{min} and δ_{max} on 30 networks with the same number of attractors m , where $m \in \{1, \dots, 20\}$.

5 DISCUSSION AND CONCLUSION

The overall outcome of our analysis is that the addition of self-loops with canalising function to RBNs affects:

- (a) the distribution of the number of attractors, mainly by gradually increasing the maximum number, moving right the peak of the density function and making the distribution flatter; in particular as the fraction of self-loops increases, density function peak becomes gradually less substantial and tails longer;
- (b.1) the **maximum** attractor robustness in two ways: it decreases with the number of attractors, but gradually increases adding self-loops if compared across BNs with the same number of attractors;
- (b.2) the **minimum** attractor robustness in two ways: it decreases with the number of attractors, and exhibits a composite behaviour if compared across BNs with the same number of attractors but different number of self-loops (it grows for BNs with few attractors and decreases for many attractors, while it is approximately steady for an intermediate number).

Given these experimental results, we claim that self-loops in BNs can positively influence their dynamic behaviour—according to the characteristics required for modelling cell differentiation—but the fraction of self-loops must be accurately chosen to guarantee the best balance between number of attractors (cell types) and attractor robustness (cell type stability). Our claim is that in the range 25–45% of nodes with a self-loop in the network, we can observe a substantial advantage in robustness without exceeding in the amount of attractors. From the analysis we conducted on the Cell Collective [34] database, we found that, among the GRNs with self-loops (54% of the networks available, as discussed in Section 2), the average value of the fraction of self-loops is 0.2100 with a standard deviation of 0.2104. Details on the distribution are shown in Figure 8. This analysis on

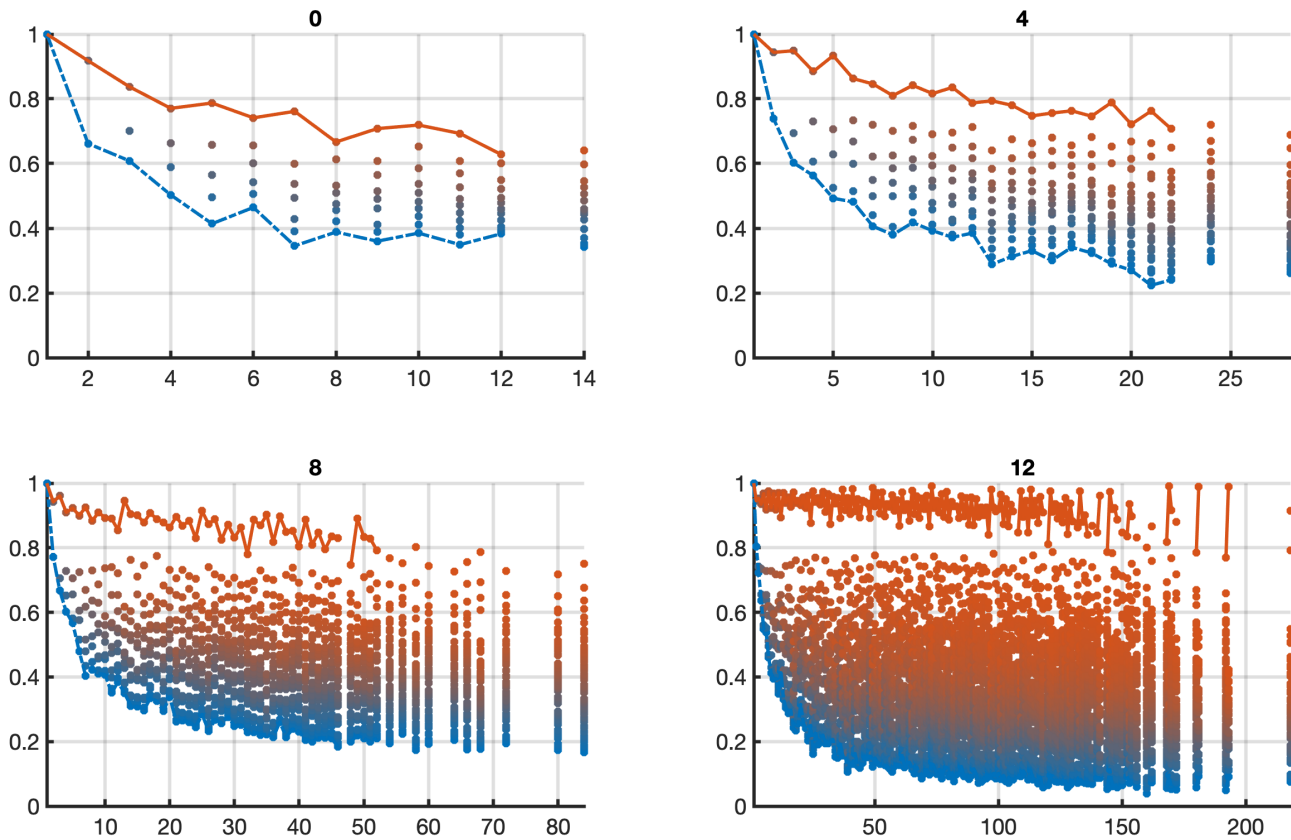


Figure 6: δ_{min} , δ_{max} and ATM diagonal values as a function of the number of attractors for CONST-OR configuration. Results are shown for $n_s \in \{0, 4, 8, 12\}$ (see title in each plot).

real networks confirms our hypothesis and leads to the conclusion that a fraction of about 30% self-loops can bring an evolutionary advantage to BN dynamic, especially once modelling cellular differentiation processes.

In particular, simulation results enable us to formulate an evolutionary hypothesis that may be tested *in silico* by means of BNs. Let us suppose that attractors—or sets of attractors—represent cell types. Our conjecture is that autoregulation may have appeared in evolution as a functional component that makes it straightforward to (i) increasing the number of attractors (i.e. cell types) without severely perturbing the other dynamical properties of the network and (ii) consolidating dynamical attractors, e.g. by increasing the robustness of some of them (in other words, to increasing the maximum attractor robustness in a BN with m attractors, a moderate rewiring adding self-loops would be a quite effective procedure). Indeed, a system is evolvable if, subject to mutations on its structure, it exhibits variability in phenotypic traits that may undergo selection. Besides this, by being quite simple and local modifications, self-loops are good candidates as mutation perturbations in evolutionary schemes. As a future work, we plan to investigate this evolutionary hypothesis.

Some questions may be raised concerning the properties of the model we studied in comparison with RBN models studied in the current literature. A first question may arise about the dynamical regime—ordered, disordered

or critical—of RBNs with self-loops.⁵ To the best of our knowledge, this property has not yet been studied so far and we are currently investigating it. However, it should be observed that in the case in which OR functions are introduced with self-loops, the canalising effects are very likely to keep the networks in an ordered regime. Moreover, one may ask to what extent the results obtained for CONST-OR and AUGM-OR differ from those that can be attained in classical RBNs in which the function of some nodes is forced to be an OR. In fact, we addressed this question in a previous work [11] and found that the two models produce strikingly different results. Therefore, the effect of self-loops in RBNs can not solely be ascribed to the specific Boolean function used, but it crucially depends on the topological feature of autoregulation.

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⁵ The topic of BNs dynamical regime is rather wide and out of the scope of this paper; we refer the interested reader to specialised literature in dynamical criticality [18], [46], [47].

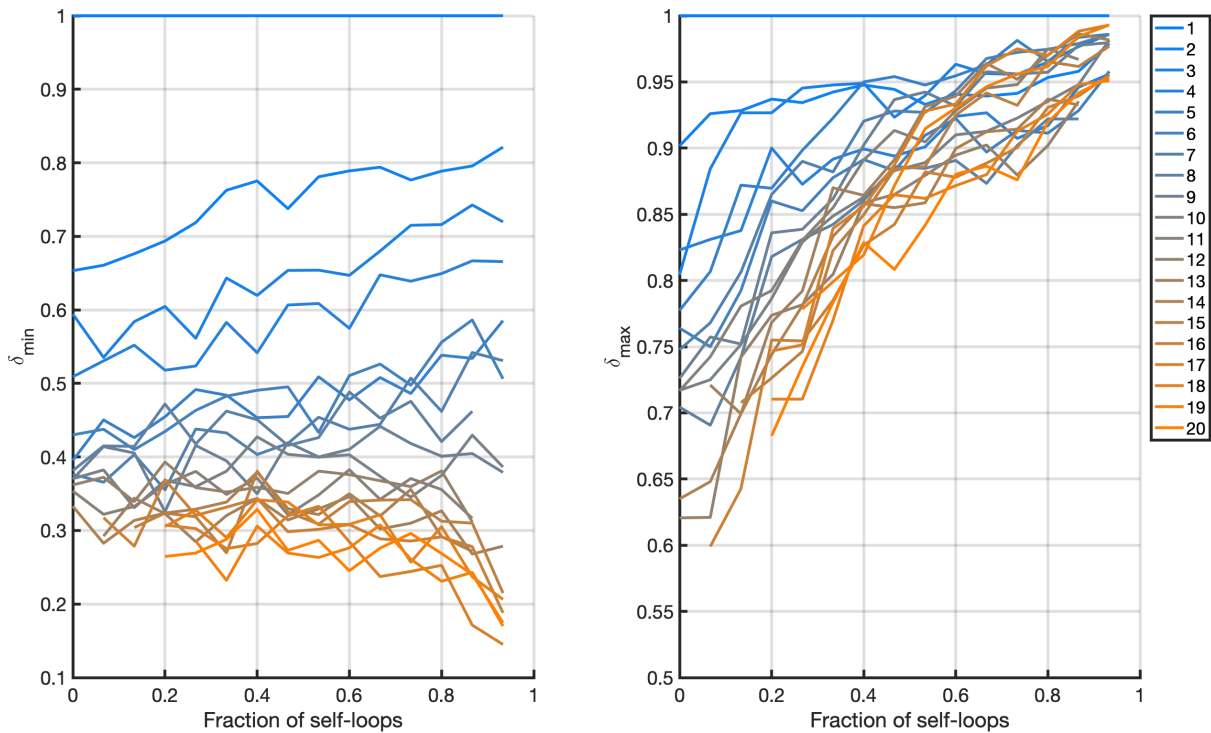


Figure 7: δ_{min} (left) and δ_{max} (right) as a function of the fraction of nodes with a self-loop for CONST-OR configuration. Each plot refers to the average values of δ_{min} (left) and δ_{max} (right) on 30 networks with the same number of attractors m , where $m \in \{1, \dots, 20\}$ (see legend).

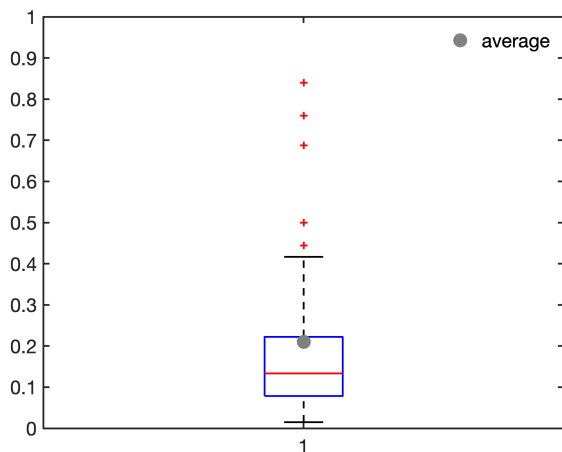


Figure 8: Distribution of the fraction of nodes with a self-loop in real networks from the Cell Collective [34] database.

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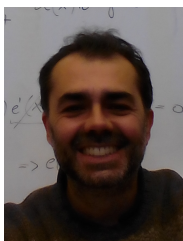
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