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“Immunetworks”, intersecting circuits and dynamics

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Abstract

This paper proposes a study of biological regulatory networks based on a multi-level strategy. Given a network, the first structural level of this strategy consists in analysing the architecture of the network interactions in order to describe it. The second dynamical level consists in relating the patterns found in the architecture to the possible dynamical behaviours of the network. It is known that circuits are the patterns that play the most important part in the dynamics of a network in the sense that they are responsible for the diversity of its asymptotic behaviours. Here, we pursue further this idea and argue that beyond the influence of underlying circuits, intersections of circuits also impact significantly on the dynamics of a network and thus need to be paid special attention to. For some genetic regulation networks involved in the control of the immune system (“immunetworks”), we show that the small number of attractors can be explained by the presence, in the underlying structures of these networks, of intersecting circuits that “inter-lock”.

Keywords: Discrete dynamical systems, biological regulatory networks, random networks, asymptotic behaviour, immune system networks.

1. Introduction

Since researches have focused on modeling genetic regulation networks by Boolean automata networks, many authors have noticed that the num-

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ber of attractors observed experimentally (*i.e.*, the number of their different possible asymptotic behaviours such as fixed or cyclic configurations of expressed and/or silent genes) is in general very small compared to the number of different network configurations: a Boolean network of size n has 2^n different configurations but only around \sqrt{n} attractors [1, 2]. Although this property is of mathematical order, it has real pertinence also from the biological point of view. Indeed, since Delbrück [3], it is common to compare differentiated cellular types controlled by genetic regulation networks with attractors of the model theoretical networks (see Appendix F). Further, Thomas [4] developed the idea that specific structural patterns known as *circuits* (*i.e.*, cyclic chains of interactions) play a crucial role in the dynamics of regulation networks in that they are responsible for the diversity in the possible asymptotic behaviours of networks.

Now, in an arbitrary network, besides the elements that belong to circuits (seen as the “core” elements), all other elements belong to *trees* (see Appendix A for details) that are either *down-trees* (*i.e.*, that have no influence on the rest of the network), either *up-trees* (*i.e.*, that undergo no influence from the rest of the network and only eventually act punctually on it by allowing or disallowing it to have its own dynamics), or trees connecting disjoint sets of intersecting circuits, only serving as information pathways. In line with dynamical systems theory, graph theory and Thomas’ ideas, we propose here to exploit these distinctions and our theoretical knowledge of the dynamics of circuits to carry out further the analysis of the relations that exist between the structural and the dynamical properties of regulation networks. We will develop and emphasise particularly the idea that *the very small number of observed attractors mentioned above can be related to the existence of intersections of circuits in networks structures*. Our analyses will be based on the hypothesis that from our knowledge of (*i*) the existence and location of underlying crucial patterns such as circuits in the architecture of a regulation network and of (*ii*) how, in theory, these patterns behave dynamically and (*iii*) how they interact, we may draw a global understanding of the dynamics of the network. Under this assumption, results from [5, 6] that enable us to predict the number of isolated and intersecting circuits, as well as the number of up- and down-trees in random networks and results from [7, 8] concerning the dynamics of (intersecting) circuits can be used to produce an estimation of the number of attractors of random networks.

With the aim of giving answers in both mathematical and biological contexts, we will focus in this paper on biological regulation networks involved

in the control of the immune system. We call these networks “immunetworks”. We will develop the idea that as well as specific structural patterns (*e.g.*, regulatory circuits, up-trees...), *interactions* between these patterns (*e.g.*, interactions between circuits due to an *intersection gene*, influences of boundary elements, such as micro-RNAs, on more central elements...) also impact considerably on the dynamics of these networks. Further, we will relate this idea to matters such as the evolution of immunetworks towards unequivocal functions, ageing, and also how to help build our knowledge of underlying gene-to-gene interactions based on a comparison between theoretical and experimental results.

We will start, in Section 2, by presenting the theoretical objects that we use to model biological regulation networks, that is, Boolean automata networks and their update schedules. Then, in next section, Section 3, we will introduce the theoretical notions of isolated and intersecting circuits and we will recall some of their dynamical properties. In Section 4, we will focus on the dynamics of concrete examples of immunetworks and finally, in Section 5 we will list perspectives of this work and propose a series of open problems and conjectures that we believe to be relevant.

2. Theoretical preliminaries

Informally, a Boolean automata network is described by two sets. The first set is the set of automata that represent the genes involved in the regulation network that is being modeled. In a Boolean automata network, automata take only two possible states: 0 or 1, *i.e.*, inactive or active. Let us note that in general automata networks, the automata may take several different integer states (ranging from state 0 representing the inactivity of the corresponding gene g to an integer state $q_g \in \mathbb{N}$ representing the highest concentration of the protein produced by gene g) [9, 10, 11]. The second set needed to describe an automata network is that of the interactions between the automata. These interactions, that are pictured by arrows (or *arcs*) between automata, represent positive and negative influences that genes have on one another via their proteins (see Appendix B for details on biological regulation networks modeling).

Now, formally, a *Boolean automata network* N is defined by a digraph (see Appendix A for details on graph theory) $G = (V, A)$ and a set \mathcal{F} of $|V| = n$ Boolean functions $f_i : \{0, 1\}^n \rightarrow \{0, 1\}$ (one for each automata $i \in V$) that are called *local transition functions*. The digraph G is called the *interaction graph* of the network N . Figure 1 pictures the two instances

of interaction graphs that we will pay special attention to in this article: (isolated) *circuits* and *intersecting circuits*. Nodes or vertices of G , *i.e.*, elements of the set V , are assimilated to the automata of N (and thus also to the genes of the real genetic network being modeled). The set A of arcs of G represents the *effective* interactions between automata: for every local transition function $f_i : \{0, 1\}^n \rightarrow \{0, 1\}$ ($i \in V$), the arc (j, i) belongs to A if and only if f_i depends on its j^{th} component. It is common practise to label by a $+$ sign and call *positive arc* any arc that conveys a positive dependency and label by a $-$ sign and call *negative arc* any arc that conveys a negative dependency (see examples in Figure 2). Thus, we say that a circuit is *positive* if it contains an even number of positive arcs and we say that it is *negative* if it contains an odd number of negative arcs. Note that in some figures (Figure 4, left for instance) originated from a biological background, positive arcs are represented by simple unlabeled arrows and negative arcs by flat-headed unlabeled arrows.

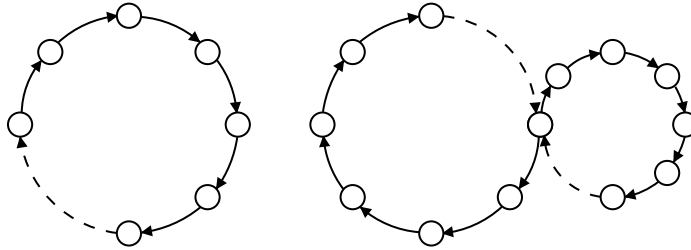


Figure 1: Two network interaction graphs: a circuit (left) and two tangentially intersecting circuits (right).

Since every one of the n automata can take one of the two states 0 and 1, the network N has 2^n different configurations (or states) which are identified with Boolean vectors $x \in \{0, 1\}^n$ whose i^{th} component x_i represents the state of automata i . In order to define the dynamics of a Boolean network N , we set the temporal space to be \mathbb{N} and write $x(t) \in \{0, 1\}^n$ to refer to the configuration of the network N at time step t . Then, the set \mathcal{F} of local transition functions together with an *update schedule* (*a.k.a. iteration mode*) specifies the transitions that the network N may perform between configurations. More precisely, each Boolean function $f_i : \{0, 1\}^n \rightarrow \{0, 1\}$ in the set \mathcal{F} maps a configuration of the network to a state of the automaton i . Thus, if $x(t) \in \{0, 1\}^n$ is the configuration of the network N at time t and if $W \subseteq V$ is the set of automata whose states are to be updated between time steps t and $t + 1$, then the new configuration of the network at time

$t + 1$ is $x(t + 1) = F_W(x(t))$ where:

$$x_i(t + 1) = \begin{cases} f_i(x(t)) & \text{if } i \in W, \\ x_i(t) & \text{otherwise.} \end{cases}$$

Note that, more generally, one can define update schedules such that, between two observations of the network configuration, at time t and then at time $t + 1$, several sets W of automata are updated sequentially. This is the case of the *block-sequential* update schedules that were introduced by Robert [9]. Indeed, any given block-sequential update schedule defines a partition of V comprised of disjoint sets $W_k \subseteq V$, $k \leq m \leq V$ of automata such that between time steps t and $t + 1$, all automata in one same set W_k are updated in parallel whereas the sets themselves are updated sequentially. In other words, at the end of each update sequence (W_1, W_2, \dots, W_m) starting at time t where $x(t)$ is the network configuration, all automata of the network have been updated exactly once and the network has taken state $x(t + 1) = F_{W_m} \circ \dots \circ F_{W_2} \circ F_{W_1}(x(t))$. Particular cases of block-sequential update schedules are the parallel update schedule (that updates all automata at once) and the $n!$ different sequential update schedules (that update automata one at a time according to a predefined sequence). In this paper, we focus on the parallel update schedule and thus the dynamics of networks will be given by:

$$\forall x = x(t) \in \{0, 1\}^n, \quad x(t + 1) = F_V(x) = (f_1(x), f_2(x), \dots, f_n(x)).$$

The dynamics of a Boolean automata network can be represented by a digraph in which nodes correspond to configurations $x(t)$ and arcs corresponds to transitions $(x(t), x(t + 1))$ between configurations (see Figure 2). This digraph is called the *transition graph* of the network. *Attractors* of a Boolean automata network are the circuits in its transition graph. The period of an attractor is the number of configurations it contains. Attractors of period 1 are usually assimilated to the sole configuration they contain and called *fixed points*. Attractors of larger periods are called *limit cycles*. The set of configurations leading towards a given attractor is called the *attraction basin* of this attractor [12, 13]. The notion of attraction basin will be of importance here because, as in [14], attraction basins will serve later as *gauges* of the robustness and plausibility of some attractors that will be considered. More precisely, we will abide by the idea that the greater the size of the attraction basin of an attractor \mathcal{A} is, the most likely is this attractor.

As mentioned above, here, we choose to study networks under the par-

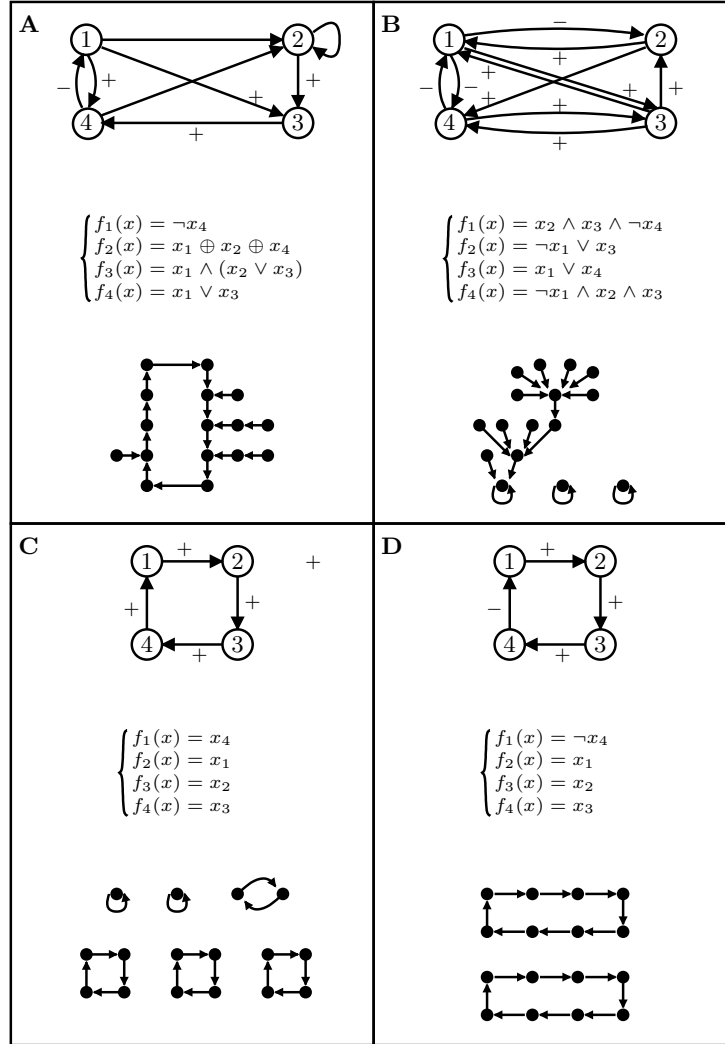


Figure 2: Four different Boolean automata networks (A, B, C, D) of size 4. In each box figures, from top to bottom, the interaction graph of the corresponding network, the set of local transition functions $\forall 1 \leq i \leq 4, f_i : \{0, 1\}^4 \rightarrow \{0, 1\}$ and a representation of the transition graph of the network. Some arcs of the interaction graph of network A are not signed. These arcs convey dependencies that are given by the local transition function f_2 of automata 2 which is defined by the XOR function. Network A has a limit cycle of period 10 whose attraction basin is composed of 6 configurations. Network B has three fixed points amongst which only one has a non empty attraction basin of size 13. Networks C and D both have circuits as interaction graphs and in agreement with results in [7, 15], they both only have attractors without attraction basins.

allel update schedule. The reason is twofold. First, from [9], we know that the dynamics of an arbitrary network N updated with an arbitrary block-sequential update schedule is equivalent to that of a network N' updated with the parallel update schedule. Thus, as far as block-sequential update schedules are concerned, from a methodological point of view, it suffices to study and understand dynamics under the parallel update schedule only. In addition, one of our main aims is to determine upper bounds on the number of attractors of some specific networks. Studies performed in [16, 17, 18] showed that, for most networks, the maximum number of attractors is obtained with the parallel update schedule. Indeed, in [19], the analysis concerning the dynamics of circuits exploiting results of [9] shows that sequentialising the updating of a network tends to make it equivalent to a network whose circuits are smaller and thus whose attractors are smaller and less numerous (see [7, 8, 15] for a combinatorial study of the dynamical behaviours of circuits).

3. Circuits and intersecting circuits

Another significant choice that we make in this article is to focus on how circuits and intersecting circuits impact on the dynamics of networks that contain them. Now, underlying circuits in networks are known since the 1980's to be responsible for the diversity in their dynamical behaviours. Indeed, in [4], Thomas formulated two conjectures concerning the importance of circuits and their relation to the dynamics of networks:

- the presence of an underlying positive circuit is a necessary condition for multistationarity (the existence of several fixed points);
- the presence of an underlying negative circuit is a necessary condition for stable oscillations (the existence of limit cycles).

Since then, these conjectures have been proven in different frameworks [20, 21, 22, 23, 24, 25, 26, 27, 28, 29]. As for intersecting circuits, we will argue here that beyond the impact that circuits have on the dynamical behaviour of a network, the interactions of circuits via their intersections also account significantly for certain dynamical properties of networks.

To do so, we will rely on the theoretical results presented in [7, 8], discussed in Appendix F, and presented in Tables 1 and 2. These results give the number of attractors of isolated circuits and of pairs of tangentially intersecting circuits in parallel. They induce possible comparisons between the asymptotic dynamics of these networks. These comparisons are summarised

in the list below. To illustrate them, let us consider the following example. Let \mathcal{C}_ℓ be a negative circuit of size $\ell = 8$, let \mathcal{C}_r be a positive circuit of size 10 and let N be the network consisting of the tangential intersection of the two circuits (as in Figure 1). Then, according to Table 1 (column 10, row $\gcd(8, 10) = 2$), N has 14 attractors. Note that similar examples with two negative circuits may easily be drawn from Table 2. Figure 3 represents one of them.

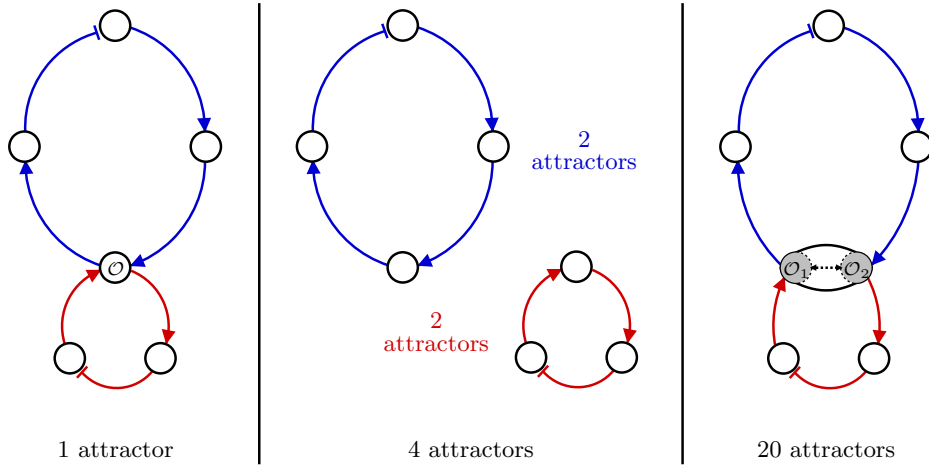


Figure 3: *Left:* A network that has one unique attractor and which is comprised of two negative circuits of respective sizes 4 and 3 intersecting on node \mathcal{O} . *Centre:* Isolated, each of these circuit has 2 attractors. Thus if they are contained both in a larger network, their reunion yields $2 \times 2 = 4$ attractors. *Right:* Ignoring the intersection in the first network (left) and focusing on the circuit that encircles both smaller circuits is equivalent to replacing node \mathcal{O} by a bipolar node $\{\mathcal{O}_1, \mathcal{O}_2\}$ or to supposing that node \mathcal{O} does not transmit fairly and synchronously the information it receives to both circuits. This yields a network with 20 attractors.

- The tangential intersection N of two circuits \mathcal{C}_ℓ and \mathcal{C}_r of respective sizes ℓ and r and respective signs s_ℓ and s_r , induces less attractors than what a circuit of size $\ell + r$ and of sign $s_\ell \times s_r$ has. In other words, the network consisting in the longest circuit encircling \mathcal{C}_ℓ and \mathcal{C}_r in N has more attractors than N . For the example introduced above, the “encircling circuit” has size $8 + 10 = 18$, is negative and according to Table 2 (last row of column T_ℓ^-), has $7286 \gg 14$ attractors.
- An intersection of circuits has less attractors than the multiplication of the number of attractors induced by each of the circuits separately. In our current example, the negative circuit of size 8, when isolated,

has 16 attractors (see last column of Table 1 or 2), the positive circuit of size 10 has 108 attractors (see last row of Table 1) and the multiplication of these numbers equals $1728 \gg 14$.

- The number of attractors induced by an intersection of circuits can be compared to the number of attractors of one of these circuits. More precisely, if both circuits are negative, then their intersection yields less attractors than the number of attractors that has the largest negative circuit when it is isolated; if one of the circuits is positive, then, the intersection has less attractors than the number of attractors of this positive circuit.

Results in [8] suggest that the three upper bounds given above are ordered from the loosest to the tightest (this is verified by the example we chose). Let us emphasise that our interest in the first bound is that it integrates the possibility of a natural perturbation that would desynchronise the behaviour of the intersection node, associating both of its inputs to the output belonging to the other circuit, as it is pictured in the right part of Figure 3.

In similar lines, let us highlight here that it is easy to derive from results in [7, 15] that positive circuits have at least twice as many attractors as negative circuits of the same size. A result of this is that fixing the state of an underlying positive circuit of size n in a network (either by intersecting it with another circuit or by submitting it to a fixed input resulting from a source node such as a micro-RNA as we will see later on, for instance) potentially has much more drastic consequences than fixing the state of an underlying negative circuit of size n , in terms of the reduction in the number of possible attractors of the network.

Now, let us consider again two tangentially intersecting circuits \mathcal{C}_ℓ and \mathcal{C}_r and let us note that imposing that one node of the network keep a fixed state may have different results according to where this node is located. If the fixed node belongs to just one of the circuits, say \mathcal{C}_ℓ , then necessarily, the state of this circuit will also become fixed. Whether or not the state of circuit \mathcal{C}_r will also become fixed depends on the strength of the action that \mathcal{C}_ℓ has on \mathcal{C}_r via the intersection. If the fixed node is the intersection node then, necessarily, both circuits lose their ability to behave dynamically as they would without the additional constraint on the intersection node: they both end up in a fixed state. Thus, extrapolating to intersecting circuits embedded in larger networks, we can suppose that some nodes in a network are theoretically much more sensitive than others in that acting on them can impact indirectly on larger parts of the network.

		Positive circuit																			
$\ell \backslash r$	r	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	T_ℓ^-	
	1	1	1	2	2	3	3	5	5	8	10	15	19	31	41	64	94	143	211	329	1
2			1	2	3	3	4	5	8	10	14	19	31	41	63	94	143	211	328	1	
3				1	3	3	6	5	8	8	15	19	33	41	64	91	143	211	332	2	
4					1	3	4	5	11	10	14	19	24	41	63	94	156	211	328	2	
5						1	5	5	8	10	26	19	31	41	64	70	143	211	329	4	
6							1	5	8	8	14	19	63	41	63	91	143	211	232	6	
7								1	8	10	15	19	31	41	158	94	143	211	329	10	
8									1	10	14	19	24	41	63	94	411	211	328	16	
9										1	15	19	33	41	64	91	143	211	1098	30	
10											1	19	31	41	63	70	143	211	328	52	
11												1	31	41	64	94	143	211	329	94	
12													1	41	63	91	156	211	232	172	
13														1	64	94	143	211	329	316	
14															1	94	143	211	328	586	
15																1	143	211	332	1096	
16																	1	211	328	2048	
17																		1	329	3856	
18																				1	7286
T_r^+		2	3	4	6	8	14	20	36	60	108	188	352	632	1182	2192	4116	7712	14602		

Table 1: Number of attractors of a negative circuit of size ℓ intersecting a positive circuit of size r . When the size ℓ of the negative circuit is larger than the size r of the positive circuit, the number of attractors equals the number given in cell $(gcd(\ell, r), r)$ according to [8]. The row T_r^+ (resp. the column T_ℓ^-) gives the total number of attractors of an isolated positive (resp. negative) circuit of size r (resp. ℓ).

In the sequel, applying all the theoretical results presented in this section to concrete examples and computing some of the bounds listed above for these examples, we will argue in particular that intersections of circuits are responsible for substantial decreases in the number of attractors induced by a couple of circuits.

4. Immunetworks

In the sequel, we call *immunetworks* biological regulatory networks involved in the control of the immune response. They are now extensively studied and numerous genes and regulations composing their interaction graphs have been identified. Let us take as example the network that controls in mice the rearrangement process of the TCRA and TCRB loci. In [30], figures the interaction graph of this complex immunetwork. It is comprised of around a hundred genes (among which about 30 are sinks and 8 are sources) and less than three hundred interactions and thus has a connectivity of about 3 (see Appendix A). A part of this network is pictured in Figure 4.

Negative circuit

$\ell \backslash r$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	T_ℓ^-
1	1														1
2	1	1													1
3	1	1	2												2
4	1	2	1	2											2
5	2	1	2	2	4										4
6	1	1	3	3	2	6									6
7	2	2	3	2	4	3	10								10
8	2	3	2	8	3	4	6	16							16
9	3	2	2	3	5	9	7	7	30						30
10	2	4	3	4	17	7	7	10	11	52					52
11	4	3	5	6	7	7	11	11	16	19	94				94
12	3	4	9	2	7	42	11	33	17	23	28	172			172
13	5	6	7	7	11	11	16	19	24	28	39	46	316		316
14	6	7	7	10	11	17	105	23	28	38	46	60	75	586	586

Table 2: Number of attractors of two negative intersecting circuits of sizes ℓ and r . The last column gives the total number T_ℓ^- of attractors of an isolated negative circuit.

In the sequel, we call this sub-network the PU.1 sub-network. It contains two negative circuits that intersect on gene PU.1: one negative circuit of size 6 that we call the GibFi circuit and another of size 2 called the Runx3 circuit. According to Table 2, the PU.1 sub-network has a unique attractor. Now, since the GibFi circuit when isolated has 6 attractors and the Runx3 circuit has one, based on Table 2, we find that the PU.1 sub-network has less attractors than what it would have in all three of the following cases which yield respectively 36, 6 and 6 attractors (and which correspond respectively to the three upper bounds presented in Section 3 and illustrated by Figure 3): (i) the intersection gene PU.1 is *bipolar* as suggested in Figure 3 (right), (ii) the two circuits are separated, (iii) only one of the two circuits, the largest, is kept. Moreover, notice the importance of this intersecting circuit that takes part in regulating the expression of the RAG-1 gene which allows to perform both the TCRB and the TCRA rearrangements at the origin of T cell lineage [31, 32, 33].

4.1. Micro-RNAs implications in immunetworks

Any regulation network may undergo several types of actions whose effect may be to enhance the network stability or to select a particular network behaviour. Amongst these actions, are those that are due to micro-RNAs, ubiquitously present in all species. Let us note that there are three different ways in which micro-RNAs can act on a regulation network: they may either act on the down-tree or sink nodes of the network, either act on the up-tree or source nodes of the network, or they can act directly on the core of

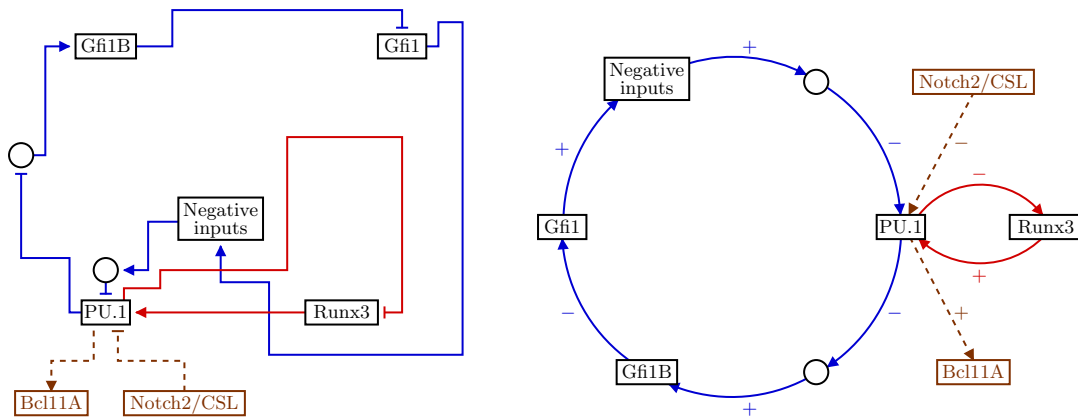


Figure 4: Tangentially intersecting circuits found in one of the regulation networks controlling the immune system of mice. On the left, a coloured snapshot extracted from the biological regulation network given in [30]; on the right, a mathematical representation of these two intersecting circuits. Dashed lines represent chains of interactions.

the network (see Appendix A). As mentioned above, these actions may all have significant impact on the dynamical behaviour of the network. Indeed, the first type of action may modify directly the output of the regulation network. The other two may have an even more significant impact since they may force underlying core circuits to remain fixed in a certain state and thus, they may have as consequence to reduce considerably the number of attractors of the network. Again, let us point out that fixing in a similar manner the state of nodes in a positive circuit particularly, also potentially has very significant impact on the number attractors of the network.

As an example, consider the immunetwork of Figure 4 (see [30] for a detailed version of the interaction graph of this network). Instances of all three actions may be recognised. Indeed, first, according to [34, 35], there are five micro-RNAs that target the sink gene *Bcl11A*. Second, nine different micro-RNAs act directly on gene *Gfi1*, and thus directly on the *GibFi* circuit. Finally, considering gene *Notch2* when bound to *CSL*, and the oriented chain of interactions that exists from the *Notch2/CSL* complex to gene *PU.1*, we find that sixteen different micro-RNAs have an indirect action on the *PU.1* sub-network through an up-tree.

4.2. Evolution of immunetworks

In [36], the authors present circuits from an immunetwork controlling the B cells differentiation (Figure 5). Interestingly, all confirmed interactions (in full lines in the figure appearing in [36]) are shared by the immunetwork

involved in the TCRA rearrangements (in blue in Figure 5). This highlights how evolving organisms have a great ability to re-use successful regulation solutions.

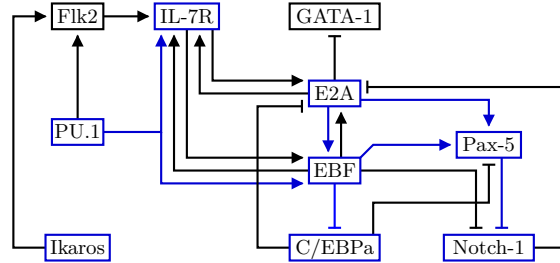


Figure 5: Genetic control common between the TCRA rearrangements (blue) and the B cells differentiation (black) [36].

More generally, if we consider biological regulatory networks in an evolutionary perspective, we can introduce a new mathematical tool to measure the increase in their structural complexity. Indeed, on one hand, we can consider that the adaptive ability of a regulatory network increases with its number of attractors and on the other hand, we can consider conversely that evolution towards a unique function devoted to tissues is that inducing the minimum ambiguity in the genetic expression. In other words, we can suppose that regulatory networks are networks that are designed so that they have maximum stability asymptotically, that is, so that they have very little attractors and when they have more than one, one of them (see for instance fixed point 2 in Figure 6 that pictures the genetic regulation network controlling the hair morphogenesis in mice) prevails through the size of its attraction basin.

As discussed above, the number of attractors of a network is tightly related to the circuits in its interaction graph. Thus, for random networks of given connectivity, the expected number of attractors may be derived from (i) the probability of there being a circuit of a given size ℓ in their interaction graph and (ii) the exact formulae (given in [7, 15]) for the number of attractors of positive and negative isolated circuits of size ℓ .

According to [37], different entropy measures may be used to describe the complexity of a random network:

- The *node entropy*, $E_{\text{node}} = -\sum_{1 \leq k \leq c \cdot n} P_k \cdot \log(P_k)$, where P_k is the probability for any node to have in-degree k (with the convention $0 \cdot \log(0) = 0$). In the case where $c \times n$ arcs are chosen uniformly at

random amongst all the possible ordered pairs of nodes, every arc has probability $\frac{c}{n}$ of existing and thus, every node has the following probability of having k incoming arcs:

$$P_k = C_n^k \cdot \left(\frac{c}{n}\right)^k \cdot \left(\frac{n-c}{n}\right)^{n-k}.$$

In this case, the node entropy is that of a binomial distribution of parameters n and $\frac{c}{n}$.

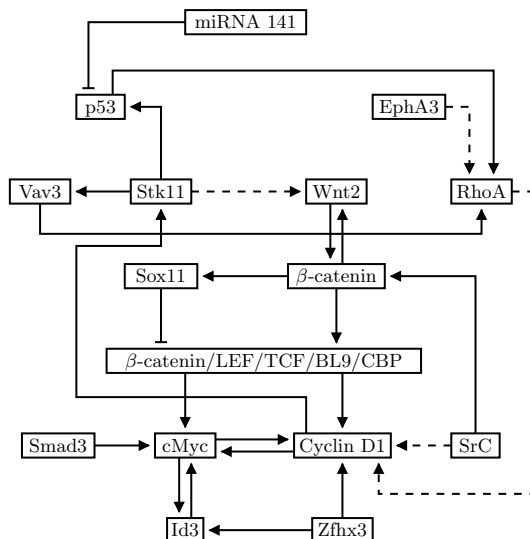
- The *attractor entropy*, $E_{\text{attractor}} = -\sum_{1 \leq s \leq 2^n} P_s \cdot \log(P_s)$, where P_s is the probability for attractors to have attraction basins of relative size s . For instance, using the attraction basins relative sizes given in Figure 6 bottom panel, one can compute the attractor entropy of the network pictured in Figure 6 top panel:

$$E_{\text{attractor}} = -[0.9966 \times \log(0.9966) + 0.0034 \times \log(0.0034)] = 9.87 \times 10^{-3}.$$

A systematic calculation of E_{node} and of $E_{\text{attractor}}$ yields an estimation of the complexification of networks that ensure one same regulation function in different species. As an example, one may consider the network regulating the cell cycle: increasing the number of sources acting on the core of this network through its up-trees (while keeping the network core unchanged) induces an increase of node entropy [38]. Let us also note that the attractor entropy of the network of Figure 6 increases considerably between the parallel update schedule (under which $E_{\text{attractor}} = 9.87 \times 10^{-3}$) and the sequential update schedule that is used for the computations (under which $E_{\text{attractor}} = 6.97 \times 10^{-2}$).

4.3. Inverse mathematical methods for immunetworks

Identifying the expression states of genes involved in immunetworks using bio-arrays data or bio-functional considerations can be of great use, we believe, in the process of inferring the underlying interactions structure of networks. Indeed, from the knowledge of the set of genes that are expressed asymptotically, inverse mathematical methods can draw a plausible set of interactions that need to exist in order to effectively yield such asymptotic states. Thus, we believe that approaches based on constraint programming and on causal model view as in [39, 40, 41, 42] may reveal themselves useful to understand the properties and perhaps even describe the underlying network architectures that are compatible with gene states that are observed in immunetworks (and believed to belong to attractors). Of course, extracting



Nature	Sequential updating		Parallel updating	
	Attractor	ABRS	Attractor	ABRS
Fixed point 1	0000000000000000	1.56%	0000000000000000	≈ 0.00%
Fixed point 2	0011111001101110	96.88%	0011111001101110	99.66%
Fixed point 3	0000010000001100	1.56%	0000010000001100	≈ 0.00%
Limit cycle 1	None	-	0011010001101100	0.34%
			0000111000001110	

Figure 6: Genetic regulation network controlling the hair morphogenesis in mice [38], its attractors and their attraction basins relative sizes (ABRS) according to the parallel and a sequential update schedule. Dashed lines represent chains of interactions.

this information requires a precise knowledge of when does the immunetwork enter its asymptotic regime, that is, its stationary or its cyclic attractors.

For example, from the data presented by [43, 44] and reproduced partially in Table 3 and Figure 7, the authors of these articles showed how to infer the expression state of important immune-related genes such as PU.1, Notch1, Notch3, GATA3, Ikaros, Runx1, Runx3, RAG1, Bcl1, Lck, ZAP70 or LAT. These genes appear as nodes of the network presented in [30] and partly represented in Figure 4. Some interactions between them are not strongly confirmed. Using inverse methods based on the knowledge of what states are taken asymptotically by the genes, it can however be checked whether these hypothetical interactions are plausible or not [39, 40, 41, 42, 45]. In addition, the likeliness of the interaction graph

constructed this way, can be checked by performing the theoretical calculations mentioned in Section 3 and confronting the results to what is observed. More precisely, finding theoretically unexpected numbers of attractors for some of the networks produced by the inverse techniques may help to slim down the set of possible network architectures by excluding those that yield unlikely asymptotic dynamics. Thus, using our knowledge of the dynamics of some key formal networks (most importantly circuits and intersecting circuits) may reveal itself very useful in the process of refining our understanding of the underlying gene-to-gene interactions that take place in a larger regulation network that we have only partial knowledge of otherwise.

For instance, from Table 2 (cell (6, 4)), we draw that the intersection at the core of the sub-network pictured in Figure 7 yields 3 attractors. If the network had been proposed with only slight variations (such as differences in the signs of some interactions or in their number), Tables 1 and 2 show that the number of attractors could have been very different, perhaps too large for the architecture to be plausible. In particular, if one circuit had been missing, the network would have had twice more attractors. If the two circuits had not been supposed to interact through the intersection gene Fyn, it would have had 4 times more attractors. To go further, considering the bipolar ability of gene Fyn, the number of attractors would be 108, *i.e.*, 36 times more, making it probably too ambiguous and unstable to be likely (supposing we abide by the hypotheses made in Section 4.2).

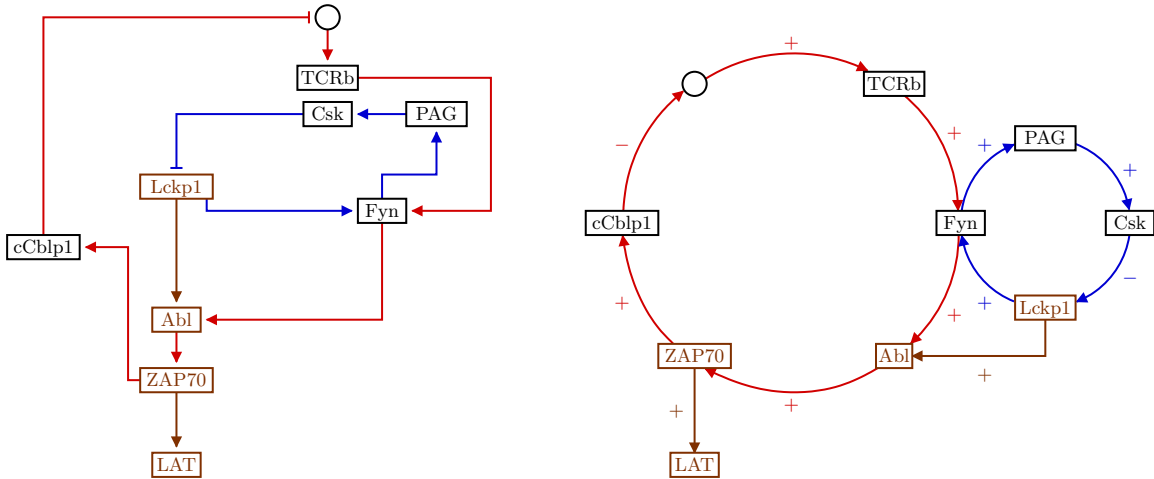


Figure 7: Part of the immunetwork of T cell activation extracted from [43].

Name	Accession	Description	Ratio		
			Brain	Heart	Kidney
Irak3	NM_028679	Interleukin-1 receptor-associated kinase 3	1.23	0.78	0.87
Icam1	NM_010493	Intercellular adhesion molecule	0.68	0.98	0.61
Tcrb-V8.2	NC_000072	T-cell receptor beta, variable 8.2	-0.66	0.99	1.41

Table 3: Over-expression of immune-related genes due to ageing extracted from [44].

4.4. Immunetworks and ageing

The functional “canalisation” of immunetworks can be seen as their property to select a unique attractor in the sense that their design either induces no more than one attractor, or they induce several but only one that is much more likely and stable than the others (see Section 4.2). The ageing process can eliminate this property, for instance, by favouring isolated circuits over intersecting ones or simply by desynchronising some of the genes that are located at the intersection of several circuits in the network interaction graph. As we have demonstrated in Section 3 and in the examples cited above, such actions can provoke a significant increase in the number of attractors. As an example, ageing induces an over-expression of gene *Irak3* in the brain (see Table 3): an over-activation of the positive circuit of size 9 to which this gene belongs [30] could lead to a substantial increase in the number of possible attractors (up to 60 according to Table 1).

In [46], another effect of ageing on the immune system is discussed. The assumption is made that the anti-ageing effect of the dietary restriction implies that there must exist signalling pathways that link nutrient sensing with an appropriate effector mechanism to enhance somatic maintenance. A candidate pathway is the Insulin/IGF-1 signalling pathway.

In the list of overlapping age-related genes, at least five genes of the genes that appear are involved in the inflammatory response: *Casp1*, *Irak3* (involved in the regulation of the T helper cells maturation, cf. Figure 8), *Cd48*, *Dock2* and *Icam1*. Also directly related to IL-1 is *Irak3* which regulates innate immunity through unknown mechanisms [49]. *Cd48* is an interleukin (IL)-3-induced activating receptor on eosinophils which may be involved in promoting allergic inflammation [50]. *Dock2* has been shown to be of key importance for lymphocyte chemotaxis [51]. Most interestingly, *Icam1* (see Table 3) is one of the proteins involved in inflammatory responses and is over-expressed in senescent cells and aged tissues. Additionally, the NF- κ B signalling cascade is crucial for the activation of human *Icam1* expression in response to inflammation [52]. More generally, it would be of great interest

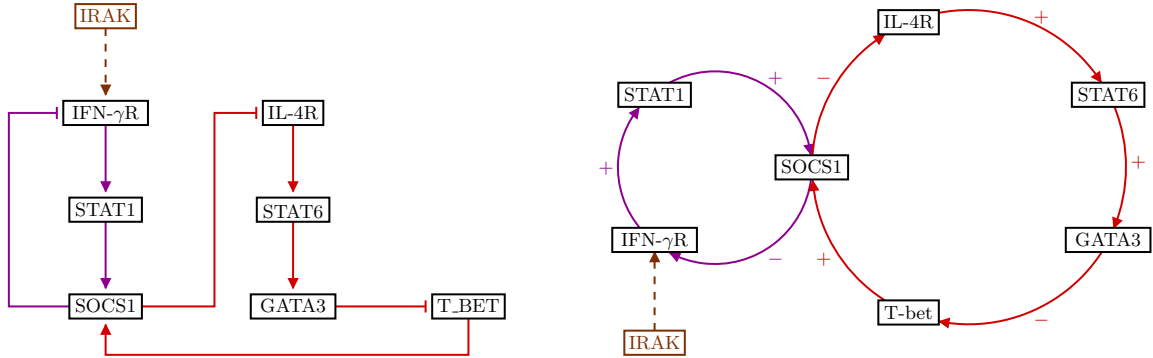


Figure 8: Immunetwork upstream the gene GATA3 regulating T helper cell maturation [47, 48]. Dashed lines represent chains of interactions.

(and thus will constitute one of the main perspectives of the present work) to elucidate the role of the immune system on ageing process acceleration either through the general inflammatory response or through more specific auto-immune pathologies, with under- or over-expressed genes involved in the immune system regulation.

As a last example, consider the regulation of the T helper cell maturation and the corresponding network pictured in Figure 8. This network exhibits one (purple) negative circuit of length 3 intersecting tangentially a (red) positive circuit of length 5. According to Table 1, due to the circuits intersection, this immunetwork has only 3 attractors, instead, for instance, of the 8 it would have, if the smallest circuit was removed. This last example is another argument in favour of the fact that the reduction of the number of attractors, *i.e.*, the evolution towards "pauci-functional" networks (controlling only few dedicated functions), could be one of the characteristics of the immune system.

4.5. Chromatin dynamics and a new update paradigm

The ultimate regulation target of an immunetwork such as those controlling antigenic loci rearrangements is the chromatin. In eukaryotes, linear DNA is associated with a protein complex of histones to constitute a compacted nucleoprotein complex, the nucleosome. The core of a nucleosome consists in 146 DNA base pairs wrapped around a histone octamer (each histone octamer contains two copies of four histones, H2A, H2B, H3, and H4). A linker DNA, roughly containing 50 base pairs, separates the nucleosome core particles. A supplementary H1 histone associates with the DNA of the nucleosomes and stabilises the fibre. This structure corresponds to

the 10-nm fibre, which further coils itself to form the 30-nm fibre. Moreover, eukaryotic inter-phase chromatin includes heterochromatin domains highly condensed and mostly transcriptionally inactive, as well as less compacted euchromatin domains containing actively expressed genes. The heterochromatin corresponds to certain euchromatic areas and might be transcriptionally inactive or active depending on the cell lineage or developmental stage.

Chromatin remodeling and sub-nuclear relocalisations of the loci coding for the TCR and Ig chains constitute prerequisites for the V(D)J rearrangements of the TCRA locus. The chromatin can be considered as a mediated epigenetic system [53, 54, 55, 56] whose structural modifications require distinctive time delays independent of gene-to-gene interactions within immunetworks.

Although many further experimental studies need to be performed in order to understand the complex role played by the chromatin state on the regulation of the immune system, we believe that some theoretical studies may help. Thus, let us propose frameworks in which these studies could be carried out.

Let us call *chrodyn genes* the genes involved in the chromatin dynamics. To account for chromatin dynamics influences, we propose to integrate chrodyn genes into our models and represent them by nodes in the interaction graphs just like any other network genes. However, obviously, these chrodyn genes need to be given somehow a *status* that distinguishes them from other genes. Indeed, we want to model the fact that when the chrodyn genes become unexpressed (consecutive to a micro-RNA inhibition, for instance), all genes depending on their being active become inactive as well and remain so until the chrodyn genes return to an active state. Thus, our model needs to give a certain primacy to interactions with chrodyn genes.

There are two ways to do this. The first way consists in representing this primacy either by heavily weighted arcs in a threshold network (see Appendix D) or more generally, by conveying it through the local transition functions of genes that depend on the state of chrodyn genes (this can be done by choosing a function that depends much more severely on its chrodyn genes components than on its other components). A second way to account for the primacy of the chrodyn genes influences is to introduce a new multi-scale update paradigm. Let us develop this idea herein general terms.

First, consider a network N and k different subsets N_1, N_2, \dots, N_k of that network. Note that the N_i 's are not necessarily disjoint. We define a digraph \mathcal{U} with k nodes, one for each N_i , and which contains the arc from

node i representing N_i to node j representing N_j if and only if the dynamics of N_j need to be activated by the nodes of N_i (or perhaps just the sink nodes of N_i , if they exist). In other words, the arc (i, j) belongs to \mathcal{U} if and only if any dynamical behaviour of N_j can only happen once the nodes of N_i are active. To be even more general and take into account more complex cases in which the dynamics of N_j depend on more than one condition (and also, possibly, to allow negative conditions such as the dynamics of N_j depend on the nodes of N_i being *inactive*), to every N_i , we associate a function h_i . Not unlike the local transition functions f_i of nodes of a network, these functions are designed so that they take the value 1 if the conditions for dynamics of N_i to take place are satisfied. Voluntarily, we leave open the question of how the h_i 's must be defined formally (besides the fact that they must convey the dependencies recorded in the arcs of \mathcal{U}).

Thus, to update the nodes in N we define a two-level update schedule. At the level of nodes inside a sub-network N_i , an update schedule (in the sense of those that have been mentioned in Section 2) is given. All sub-networks need not be given the same update schedule (for the sake of simplicity, we may however again choose the parallel update schedule for all of them). This way, *letting the dynamics of N_i take place* consists simply in updating the nodes of N_i according to its update schedule (as if it were an isolated network just like all those that have been considered until this section). Then, with respect to the constraints recorded in the arcs of \mathcal{U} , a second update schedule, at the higher level of the sub-networks themselves, specifies when each sub-network is to be *updated*, that is, when its dynamics become free to take place. Following the suggestion made above, a natural way to do this is to allow the dynamics of any sub-network N_i to take place as long as its h_i value equals 1 and freeze (and perhaps *re-initialise* it in a way that needs to be defined) as soon as h_i takes the value 0.

Besides its obvious need for some additional formal precisions, the two-scale update schedule we have just introduced adds a level of abstraction in which the *statuses* or *roles* of different sub-networks may be defined using the new digraph \mathcal{U} . This can be exploited to highlight the precedence that some sub-networks, such as those that represent the set of chrodyn genes, have on other sub-networks. In addition, the higher level part of the update schedule with the definition (to be given) of the h_i functions may allow the possibility to take into account different time scales under which sub-networks evolve. They may in particular produce a way to model time delay factors such as the decay of gene products. This way, it is possible to impose that a sub-network N_j become active only after certain genes have been active for a

long enough period of time and conversely, that N_j become inactive if the genes it relies on have been inactive for a certain amount of time.

5. Perspectives

We end this paper with some suggestions of future theoretical studies that are needed by the present state of knowledge on asymptotic dynamical behaviours of immunetworks. We express these perspectives in the form of four conjectures that have been verified in many examples but that remain to be rigorously proven and one open problem.

Conjecture 1. We conjecture that the impact of circuits intersections as described in [7] and [8] (in which the circuits considered are supposed to intersect tangentially as well as to be isolated from any environment), can be generalised to arbitrary types of circuits intersections embedded in larger interaction graphs. More precisely, the intersection of two circuits induces three possible behaviours:

- either both circuits are locked (possibly interlocked) and the states of their nodes remain fixed;
- either one of them is locked and the states of its nodes remain fixed while the other is free to behave as it would if it were alone;
- or both circuits manage to cycle together.

We conjecture that in all three cases, the number of attractors induced by this intersection is smaller than the number of attractors we would obtain if only one circuit was kept (possibly the circuit encircling the intersecting circuits, in the third case). We also conjecture that attractor periods tend to diminish, when circuits are made to intersect.

As an illustration of Conjecture 1, consider the network pictured in Figure 9 (with the model of threshold Boolean automata networks, see Appendix D). It has only two attractors (one of period 7 and one fixed point). The largest circuit encircling the two intersecting circuits of sizes 5 and 7 has size 8 and is positive. According to the last row of Table 1, if it were isolated, this circuit would have 36 attractors (all of which have a period that is smaller than 8). Another illustration of Conjecture 1 is the network controlling the hair morphogenesis in mice mentioned above (see Figure 6). In this network, when the two intersecting circuits ((cMyc, Id3) and (cMyc, cyclin D1)) are free from constraints imposed by the rest of the network

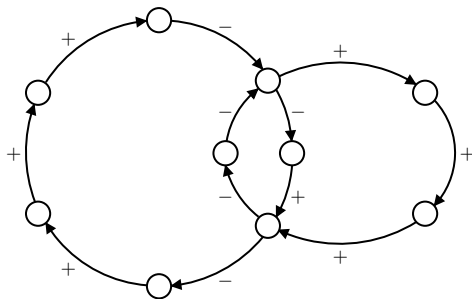


Figure 9: A network, modeled by a threshold Boolean automata network, composed of a negative circuit of size 7 intersecting a positive circuit of size 5 with two common nodes. Positive (resp. negative) arcs have weights equal to $+2$ (resp. -2). Nodes whose sole incoming arc is a positive arc have a threshold equal to $+1$; all other nodes have a threshold equal to -1 .

(*i.e.*, when the network is not in the attraction basin of the first fixed point in which all nodes are inactive), then the network has three attractors which correspond to the three attractors (two fixed points and one limit cycle of period 2) of an isolated positive circuit of size 2 (see Table 1, last row).

Conjecture 2. The number of attractors of any Boolean automata network is bounded by the number of attractors of the same network in which all strongly connected components have been replaced by a single circuit. This conjecture follows directly from Conjecture 1.

Conjecture 3. If N is a network whose underlying interaction graph G is strongly connected, then, N has a unique attractor which is a fixed point if and only if G contains a negative circuit whose size divides the size of a positive circuit in G . By the results reported in Table 1, if G is reduced to just two tangentially intersecting circuits, then this conjecture is satisfied.

Conjecture 4. According to [57] and [58], isolated circuits have a conserved energy function and thus can be called Hamiltonian systems. We conjecture that this property remains true for intersecting circuits.

Open problem. Finally, let us address the question of the choice of the most realistic way of updating the states of elements in biological regulation networks. This question is very hard in the sense that we have almost no biological information concerning any regulation clock. Indeed, biologists argue that the updating of the elements of a regulation network is rather unlikely to be perfectly synchronous or perfectly asynchronous. In addition, it is highly unlikely that *all* the genes belonging to a set of interacting

genes that form a network follow the same time delays and constraints. In Section 4.5, we discussed the case of the chrodyn genes and proposed a two-scale updating solution to take into account this last problem concerning the possibility of different time scales. As for the first problem of allowing both synchronous and asynchronous updates, several solutions have also already been proposed. The first solution are the well-known *block-sequential update schedules* introduced by Robert [9] and mentioned at the beginning of this article. Further, inspired by Robert’s update schedules, the authors of [16] defined *block-centred update schedules*. This new definition adds the possibility to observe the network state each time a node or a set (*a.k.a.* a block) of nodes changes states, by opposition to each time every node of the network has been updated exactly once. Another solution (to both problems) called *general block-parallel update schedules* was proposed in [59]. These update schedules consist in defining blocks in which, contrary to block-sequential update schedules, nodes are updated sequentially while the blocks themselves are updated in parallel. It was proven in [59] that, when the smallest blocks “wait for” the other blocks so that no block is updated more often than another, then block-parallel update schedules are equivalent to block-sequential update schedules. However, if blocks are allowed to evolve independently, we obtain a new kind of update schedule that allows the different blocks to have their own period of update. Note that one of the main differences between this update schedule and the updating method proposed earlier to take into account chromatin dynamics (see Section 4.5) is that, here, disjoint blocks of nodes evolve in parallel. Thus, the blocks have their own time constraints but can impact on each other in a more continuous manner. Note also that general block-parallel update schedules just like block-sequential update schedules and block-centred update schedules, can be integrated in the two-level updating mode proposed in Section 4.5 (at the level of the nodes of each sub-network).

Now, we believe that further theoretical studies of the solutions presented above can lead to understand the impact of update schedules on the dynamics of networks. We also believe that extending these solutions into more and more realistic (but computable) updating methods could be of great help to solve the following triplet of inverse problems, even partially or at least for specific instances of networks:

1. What is the minimal information that one needs to know concerning the interaction and transition graphs in order to be able to infer how nodes of the network are updated?
2. What is the minimal information that one needs to know concerning

the transition graph and the update schedule in order to re-construct the interaction graph of the network?

3. What is the minimal information that one needs to know concerning the update schedule and the interaction graph in order to re-construct the dynamics of the network?

6. Conclusion

We have observed that the architectures of biological regulation networks involved in the control of the immune system (immunetworks) often contain circuits that interact through their intersections. Considering like Delbrück [3] that attractors of theoretical networks represent the different cellular types controlled by the genetic regulation networks, we have focused on the number of attractors of immunetworks which represent, together with the diversity of the T cells receptors repertoire, an important aspect of the ability of the immune system to deal with exogenous aggressions. More precisely, we have emphasised the importance of the role of intersections on the dynamics of these networks and have argued that they are responsible for the fact that the number of attractors is much smaller than what one may expect to find by focusing solely and separately on the underlying circuits in the network interactions architecture. Indeed, counting the number of circuits, considering the number of attractors each one would yield if it were isolated and multiplying all these numbers produces a much higher number of attractors than what is effectively observed. Therefore, intersections of structural patterns (such as circuits) are crucial and may explain the mono- or *pauci*-functional character of the different types of immunocompetent cells. Now, our discussion has been based on our knowledge of the dynamics of circuits that intersect tangentially (i.e., that only have one node in common). Pursuing further the idea that intersections cause substantial decreases in the number of attractors, we may expect that any intersection that is not tangential (*a fortiori* that expands over several genes), will have an even more severe diminishing impact on the number of attractors. As a consequence, we believe that our argumentation still holds for such kinds of circuits intersections. However, in addition to the research perspectives that have been described earlier in this article, we believe that a formal, exhaustive study of their dynamics as was done for tangential circuits intersections would still be useful to perform more precise calculations and draw tighter bounds. For these theoretical studies, immunetworks will constitute a dedicated application.

Acknowledgements

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Appendix A. Graph theory

Appendix A.1. Graph related definitions

A *digraph* G is a couple (V, A) , where V is a set of *nodes* (or *vertices*) and A is a set of ordered pairs of nodes (or *arcs*) that represent *interactions* between nodes. If the elements of A are unordered pairs of nodes then they are rather called *edges* and G is called an *undirected graph*.

The set of arcs ending (resp. starting) in a node $v \in V$ is denoted by $\Gamma^-(v)$ (resp. $\Gamma^+(v)$). The cardinal of this set is called the *in-degree* (resp. *out-degree*) of node v and is denoted by $deg^-(v)$ (resp. $deg^+(v)$). When all nodes in a digraph have the same in- and out-degree equal to k , this digraph is said to be *k-regular*. Moreover, a node v with in-degree (resp. out-degree) 0 is called a *source* (resp. *sink*). The *connectedness* of a digraph (resp. of an undirected graph without loops $(v, v) \in A$) equals $C = \frac{|A|}{n^2}$ (resp. $C = \frac{2 \cdot |A|}{n \cdot (n-1)}$) where $n = |V|$. The *connectivity* c [1] is the average in-degree which also equals $n \cdot C$ (resp. $\frac{(n-1) \cdot C}{2}$).

In a digraph (resp. an undirected graph), a *path* (resp. a *chain*) is an ordered sequence of arcs (resp. of edges) in which the end-node of an arc (resp. an edge) is the start-node of the following one. A path is called a *circuit* if the end-node of its last arc equals the start-node of its first arc. The undirected version of a circuit is called a *cycle*. Moreover, a digraph is *strongly connected* if it contains a path from u to v and a path from v to u for every of its nodes u and v . The *strongly connected components* (denoted in the sequel by SCCs for short) of a digraph are its maximal strongly connected sub-digraphs. SCCs that contain only one node are called *trivial SCCs*. Conversely, SCCs that contain more than one node are called *non-trivial SCCs*. The digraph in which every SCC has been replaced by one node is the *reduced digraph* of the original digraph. The *core* of a digraph is defined as the sub-digraph containing all non-trivial SCCs as well as all paths between them (*i.e.* the original digraph from which the *up-trees* (resp. *down-trees*) made of the descendants of sources (resp. of ascendants of sinks) which are not in non-trivial SCCs have been removed). The *centre* of a

digraph is defined as the set of nodes u whose eccentricity (*i.e.*, the maximal length of the shortest paths from node u to all other nodes) is minimal. The *boundary* of a digraph is the set of its sources.

A *weighted digraph* is a digraph in which a real number is assigned to every arc. This number, called the *weight* of the arc, can be used to precise the type or intensity of the regulation (repression/inhibition or induction/activation) performed by the start-node of the arc on the end-node. A *signed digraph* is a weighted digraph with weights valued in $\{-1, 0, 1\}$.

Appendix A.2. Random graphs structure

Let us focus on how one may build the underlying digraph representing the architecture of a random network. Assuming we want the digraph to have a given connectivity c , it is possible, with respect to this constraint, to choose the arcs according to various probability distributions [60, 61]. For instance, one may choose uniformly at random an undirected d -regular graph in the set of all undirected d -regular graphs. In [62], Meringer gave an efficient algorithm to generate and count such graphs for small values of d . Now, if d is taken to be equal to $\frac{3 \cdot c}{2}$, then an undirected d -regular graph G with $m = \frac{n \cdot d}{2}$ edges is obtained this way. Edges of G can then be turned into arcs, by choosing uniformly between one orientation, the other or both. This yields digraphs of connectivity c [26, 57].

To go further and close this discussion, let us evoke that we can obtain a good insight of the nature of circuits contained in digraphs constructed this way. Let X_s denote the random variable representing the number of cycles of length s in a random d -regular undirected graph of size n . Supposing the degree $d = d(n)$ is allowed to increase with n , from [6, 63, 64], we know that the variables X_s are asymptotically distributed as independent Poisson variables with mean $\frac{(d-1)^s}{2 \cdot s} = \frac{(3 \cdot c - 2)^s}{(s \cdot 2^{s+1})}$ (which equals $\frac{2^{s-1}}{s}$ when $d = 3$ and $c = 2$) and with maximal circuit size $s_{\max} = s_{\max}(n)$ satisfying $(d-1)^{2 \cdot s_{\max} - 1} = o(n)$. For instance, if $d = 3$ and $c = 2$, then $s_{\max} = \frac{\log_2[o(n)] + 1}{2}$. Consequently, if $n = 22 \cdot 10^3 \approx 2^{14.5}$ (the size of the human genome), taking $o(n) = n^{\frac{2}{3}}$, we obtain that circuits have sizes less than $s_{\max} = 5$.

Appendix B. Biological regulation networks modeling

The theory of biological regulatory networks [1, 3, 4, 65] was born in parallel to the theory of neural networks [66, 67]. It is now an extensive research topic which aims at interpreting the "omic" data from bio-array devices. Biological regulatory networks are made of elements (genes, proteins,

neurons. . .) that interact. In order to represent the interaction structure of these networks, the main tool that is used is called *interaction graphs* (see Appendix A for generalities on graph theory). These digraphs are signed digraphs whose arcs represent positive or negative influences that are exerted by some biological elements of the network (represented by the start-nodes of the arcs) onto others (represented by the end-nodes of the arcs). *Positive arcs* represent activations (or inductions) whereas *negative arcs* represent inhibitions (or repressions). For instance, in many biological regulatory networks, some micro-RNAs (represented by grey nodes in Figure B.10, for instance) act as inhibiting sources in the corresponding interaction graph, that is, they act as inhibiting upper nodes belonging to the up-trees that converge towards the “core” of the network (in the graph sense defined in Appendix A). Inhibitions can also be exerted by endogenous repressors lying in the core of the network and thus belonging to regulating circuits. In the network represented in Figure B.10, for instance, gene 0 acts on gene 1 which acts on gene 2 . . . which acts on gene 5 which acts on the first gene 0. This circular sequence of interacting genes is called a *circuit* in graph theory. The core of this network contains two circuits that are both supposed to be negative (*i.e.*, both have an odd number of inhibitions). The remaining nodes of the network correspond to the up- and down-trees.

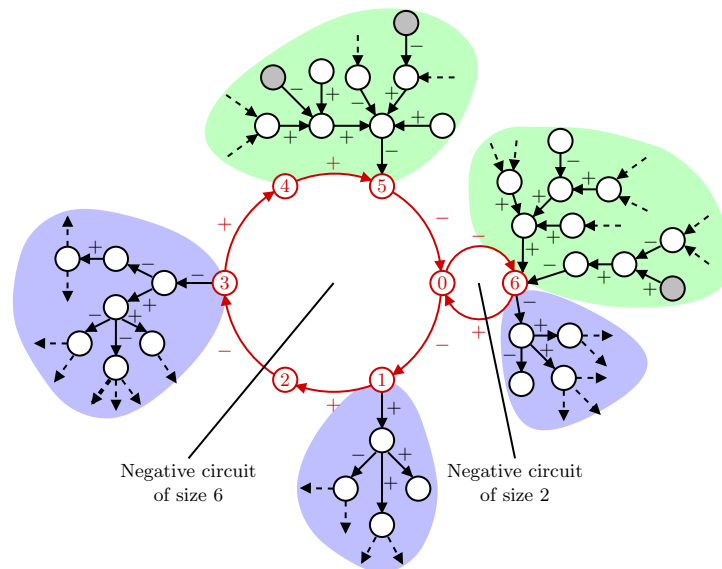


Figure B.10: Conceptual regulatory network containing two negative intersecting circuits (in red) sharing node 0: a circuit of length 6 and a circuit of length 2. The two intersecting circuits are regulated by up-trees (in green) and tend to control down-trees (in blue).

Appendix C. Kauffman Boolean networks and random Boolean networks

A *Kauffman Boolean network* [1] is a Boolean automata network in which each gene (or automata¹) i is the end-point of k arcs coming from k genes i_1, \dots, i_k (possibly including gene i itself). These k genes are called the regulators of i . In other words, the underlying interaction graph of the network is k -regular. The state of gene i is updated according to a specific rule $x_i(t+1) = f_i(x_{i_1}(t), \dots, x_{i_k}(t))$, where $x_{i_j}(t)$ is the state of regulator i_j of i at time t . The local transition function f_i of gene i is a Boolean function that can be represented by a truth table that lists its outputs for each of the possible sets of input values. Here, contrary to the formalism adopted in the body of this paper (in Section 2), f_i does not take as input the entire state of the network. Instead, it takes only as input those values gene i effectively depends on. Thus, a function f_i with k variables can receive 2^k different sets of input values. Consequently, there are 2^{2^k} different possible functions f_i .

Since Kauffman introduced this eponymous model at the end of the 1960's, numerous studies (see [68, 69, 70] for instance) have worked on generalisations of it. *Random Boolean networks* constitute one of these generalisations. The main difference between these networks and Kauffman networks is that they are not k -regular in general but are constructed randomly according to a given connectivity c , *i.e.*, *on average* their in-degree is c . Consequently, all update rules of nodes do not necessarily have the same arity. Given a random interaction graph of size n and connectivity c , we can define a random Boolean network of size n by choosing uniformly at random the local update rule for every node i of in-degree k_i amongst the $2^{2^{k_i}}$ Boolean functions of arity k_i .

When $1 \leq c \leq 2$, the number of possible limit cycles has recently been proven to be in general super-polynomial with respect to n [68, 69, 70, 71, 72].

Appendix D. Threshold networks

The interaction graph associated to a *threshold Boolean network* [66] is a weighted digraph in which the weight $w_{i,j}$ on arc (j, i) represents the intensity of the influence that gene j has on gene i , that is, the action gene j has on gene i through the protein it expresses. More precisely, for any couple of genes (j, i) , the weight $w_{i,j}$ can be positive (in the case gene j

¹From now on, we abuse language and use indifferently the words *genes* and *automata*.

induces the expression of gene i), negative (in the case gene j represses the expression of gene i) or null (in the case gene j has no influence on gene i and the arc (j, i) does not belong to the interaction graph). Thus, a threshold Boolean network is defined by an *interaction matrix* W of dimension $n \times n$ whose coefficients are the $w_{i,j}$'s. In addition, to every gene i is given an activation threshold θ_i that represents the minimal interaction potential needed to activate gene i . The Boolean vector whose coefficients are the activation thresholds θ_i , for all i belonging to the interaction graph, is called the *threshold vector* of the network and is denoted by Θ . Then, the update rule for every gene i is defined as:

$$x_i(t+1) = H\left(\sum_{j \in \{1, \dots, n\}} w_{i,j} \cdot x_j(t) - \theta_i\right),$$

where $\sum_{j \in \{1, \dots, n\}} w_{i,j} \cdot x_j(t)$ is called the *interaction potential* of gene i at time t and H is the Heaviside function satisfying $H(x) = \begin{cases} 0 & \text{if } x \leq 0, \\ 1 & \text{otherwise.} \end{cases}$

In order to illustrate the relevance of studies on this kind of model, we introduce some results concerning the dynamics of threshold networks and their underlying circuits. In these particular networks, configurations are elements of $\{-1, 1\}^n$. Note that configurations $y(t) \in \{-1, 1\}^n$ can be obtained from Boolean networks with configurations $x(t) \in \{0, 1\}^n$, and vice versa, by using the relation $y_i(t) = 2 \cdot x_i(t) - 1$, where $x_i(t) \in \{0, 1\}^n$. The hypotheses for which the following results hold are:

- the update schedule is parallel;
- the networks are strongly connected and thus contain at least one circuit;
- all sources maintain their initial state;
- the interaction matrices $W_{n \times n}$ have a quasi-minimal structure. This means that for all nodes i and j such that $i \neq j$, the arc (j, i) belongs to the interaction graph G if and only if there exists $x \in \{-1, 1\}^n$ such that $H(\sum_k w_{i,k} \cdot x_k - \theta_i) \neq H(\sum_{k \neq j} w_{i,k} \cdot x_k - \theta_i)$. This implies in particular that $\forall i \in \{1, \dots, n\}$, $-\sum_k |w_{i,k}| < \theta_i \leq \sum_k |w_{i,k}|$.

Under these hypotheses, the authors of [45, 73, 74] proved the following results:

Theorem 1. *If all cycles of the undirected version of G are positive then there exists a vector $x = (x_1, \dots, x_n) \in \{-1, 1\}^n$ such that x and $-x$ are fixed points.*

Theorem 2. *If all circuits of G are negative then all attractors are limit cycles.*

Theorem 3. *If m is the total number of positive circuits of G then the number of fixed points is less than 2^m . This upper bound is reached if and only if, for all circuits C , there does not exist an arc (k, i) ending in C , with k out of C (i.e., there is no source for C).*

Theorem 4. *If there are n arcs (as many as there are nodes) then the network has a fixed point x if and only if G contains a positive circuit (in this case, x and $-x$ are both fixed points).*

Appendix E. Relationships between the different formalisms

First, as it has been explained in Appendix D, in certain applications [27, 45, 67, 73, 74], the state 0 is replaced by -1 by a simple change of variable.

Obviously Kauffman and threshold networks can be expressed as particular instances of general Boolean networks. For the particular case of circuits that we pay special attention to in this paper, the converse also holds. Indeed, a Boolean network whose underlying interaction graph is a circuit, is 1-regular. Thus, it is a Kauffman network. In addition, such a Boolean network can also be expressed in terms of thresholds since the following expressions hold (respectively for a gene i activated by its predecessor and for a gene j repressed by its predecessor):

$$\begin{aligned} x_i(t+1) &= f_i(x_{i-1}(t)) = x_{i-1}(t) = H(x_{i-1}(t) - 0) \quad \text{and} \\ x_j(t+1) &= f_j(x_{j-1}(t)) = \neg x_{j-1}(t) = H(-x_{j-1}(t) + 1). \end{aligned}$$

It can also be checked that Boolean networks with locally monotonous transition functions (i.e., such that the corresponding arcs may be signed either by a $+$ sign or a $-$ sign unlike function f_2 in Figure 2.A) of arity 2 can also be put in terms of threshold functions. As a consequence, just as simple circuits, intersecting circuits can be considered also as instances of threshold automata networks.

Appendix F. Biological importance of attractors

A huge mathematical effort has been done to identify the sources of the attractor multiplicity, known to be closely related to the number of positive circuits underlying regulatory networks [4, 15, 22, 23, 57, 75, 76, 77, 78] as well as to understand the causes of attractor uniqueness. Both these problems are of high interest if we want to explain the number of differentiated functions (around 300 for the human being [57]) as well as the presence of some unique functions that are devoted to one tissue.

In [7, 8], the dynamical behaviour of isolated and tangentially intersecting circuits has been studied. General combinatorial properties of the asymptotic dynamical behaviours of these specific networks have been proven theoretically. Concerning isolated circuits (positive as well as negative), explicit formulae of the number of attractors of period p and of the total number of attractors of a circuit of size n have been given. About intersecting circuits for instance, if the signs of the circuits are equal, the period of an attractor divides the sum of the lengths of the two circuits. Moreover, two positive intersecting circuits admit two fixed points whereas a positive circuit intersecting a negative one (resp. two negative intersecting circuits) admits only one fixed point (resp. does not admit any fixed point). Other combinatorial results, such as the number of attractors of a specific period for two intersecting negative circuits, have been obtained theoretically. From these works which led to a precise knowledge of how isolated and intersecting circuits work dynamically, were derived Tables 1 and 2. The importance of these results lies in that they imply that intersecting circuits act as reducers of the number of attractors. To go further, in specific frameworks, they allow to explain the bijective relation between function and tissue.

A relevant example showing the biological relevance of studying attractors is that of the network which regulates the *Arabidopsis thaliana* flower morphogenesis [27, 79, 80]. This network can be modeled by a threshold Boolean automata network. It is then represented by an interaction matrix W of dimension 12×12 with only 25 non zero coefficients (see Figure F.11). Structurally composed of seven positive circuits (one of size 1 and six of size 2 that intersect pairwise) and two negative circuits (one of size 3 and one of size 4 that share two arcs, *i.e.*, three nodes), this network has six fixed points amongst which four represent floral tissues (sepals, petals, stamens and carpels), one corresponds to a tissue which is in the plant but not in the flower (inflorescence) and one has never been observed neither in nature nor by experimentation (mutant). In [14], the authors have emphasised mathematical relationships between some structural features of the network, its

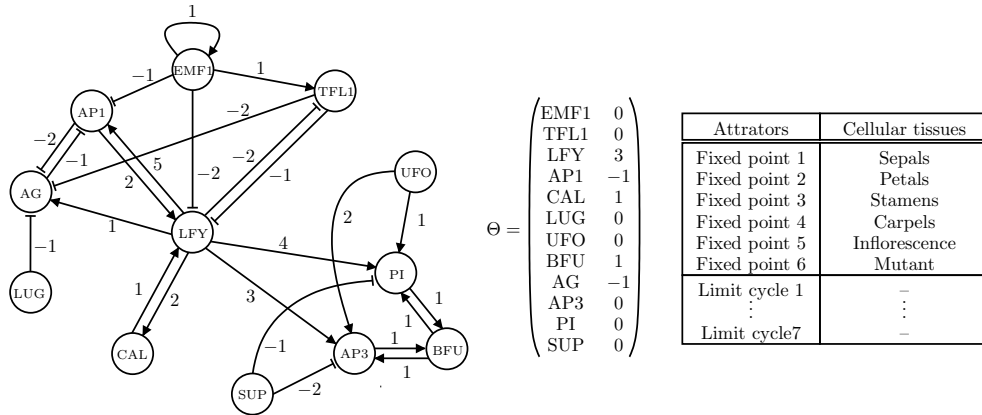


Figure F.11: Gene regulation network of the floral morphogenesis of the plant *Arabidopsis thaliana* and its attractors with the parallel iteration mode [79].

attractors and the physiological functions of the plant that ensure its flowering. Other studies [81, 82] about the floral morphogenesis of *Arabidopsis thaliana*, closer to the domain of statistical physics, have emphasised links between networks dynamics and cellular differentiation.

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