Therapeutic target discovery using Boolean network attractors: updates from kali

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Abstract

In a previous article, an algorithm for discovering therapeutic targets in Boolean networks modeling disease mechanisms was introduced. In the present article, the updates made on this algorithm, named kali, are described. These updates are: i) the possibility to work on asynchronous Boolean networks, ii) a smarter search for therapeutic targets, and iii) the possibility to use multivalued logic. kali assumes that the attractors of a dynamical system correspond to the phenotypes of the modeled biological system. Given a logical model of a pathophysiology, either Boolean or multivalued, kali searches for which biological components should be therapeutically disturbed in order to reduce the reachability of the attractors associated with pathological phenotypes, thus reducing the likeliness of pathological phenotypes. kali is illustrated on a simple example network and shows that it can find therapeutic targets able to reduce the likeliness of pathological phenotypes. However, like any computational tool, kali can predict but can not replace human expertise: it is an aid for coping with the complexity of biological systems.

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Contents

1 Introduction

In a previous article, an algorithm for *in silico* therapeutic target discovery was presented in its first version [1]. In the present article, updates made on this algorithm, named kali, are described. The complete background was introduced in the previous article whose some important concepts are recalled in *Appendix 5.1* page 13.

kali still belongs to the logic-based modeling formalism [2–4], mainly Boolean networks [5, 6], and keeps its original goal: searching for therapeutic interventions aimed at healing the supplied pathologically disturbed biological network. Such a network is intended to model the mechanisms of the studied disease and is on what kali operates. In this work, therapeutic interventions are combinations of targets, these combinations being named bullets. Targets are components of the considered network, such as enzymes or transcription factors, and can be subjected to inhibitions or activations. This is what bullets specify: which targets and which action to apply on them.

The pivotal assumption on which kali is based is that the attractors of a dynamical system, such as a Boolean network, are associated with the phenotypes of the modeled biological system, such as a signaling pathway. In other words: attractors model phenotypes [7]. This assumption was successfully used in several works [8–14] and makes sense since the steady states of a dynamical system, the attractors, should correspond to the steady states of the modeled biological system, the phenotypes.

In the mean time, various works using logical modeling with applications in therapeutic innovation was published. For example, Melody Morris and colleagues used fuzzy logic through their constrained fuzzy logic formalism [15]. This formalism aims at giving more quantitative abilities to logical models. They applied this approach on a molecular interaction network dedicated to hepatocellular carcinoma microenvironment. They successfully predicted the impact of several combinations of kinase inhibitors on key transcription factors. An other example is the work of Hyunho Chu and colleagues [16]. They built a molecular interaction network involved in colorectal tumorigenesis and studied its dynamics, particularly its attractors and their basins, with stochastic Boolean modeling. They highlighted what they named the flickering, that is the displacement of the system from one basin to an other one due to stochastic noise. They suggest that flickering is involved in pushing the system from a physiological state to a pathological one during colorectal tumorigenesis.

Concerning kali, three updates were done: i) adding the possibility to work on asynchronous Boolean networks, ii) making the selection of therapeutic bullets smarter, and iii) adding the possibility to use multivalued logic.

1.1 Handling asynchronous updating

To compute the behavior of a discrete dynamical system such as a Boolean network, its variables have to be iteratively updated. These iterative updates, also named time steps, can be made synchronously or not [17]. If all the variables are simultaneously updated at each iteration then the network is synchronous, otherwise it is asynchronous.

Compared to asynchronous updating, synchronous updating is easier to compute. However, when a biological network is simulated synchronously, it is as-

sumed that all its components evolve simultaneously, which can be not acceptable depending on what is modeled. The asynchronous updating is frequently built so that only one randomly selected variable is updated at each iteration. This allows to capture two important features: i) biological entities do not necessarily evolve simultaneously, and ii) noise due to randomness can affect when a biological interaction takes place [18–20]. This is particularly true at the molecular scale, such as with signaling pathways, where macromolecular crowding and Brownian motion can impact the firing of biochemical reactions [21].

Therefore, the choice between a synchronous or an asynchronous updating may depend on the model, the computational resources and the acceptability of the assumptions implied by synchrony. Knowing that the luxury is to have the choice, kali can now use synchronous and asynchronous updating.

1.2 Managing basin sizes for therapeutic purposes

Until now, kali requires therapeutic bullets to remove all the attractors associated with pathological phenotypes, named pathological attractors. This criterion for selecting therapeutic bullets is somewhat drastic. A smoother criterion should enable to consider more targeting strategies, and then more possibilities for counteracting diseases. However, it could also unravel less effective therapeutic bullets, but being too demanding potentially leads to no results and loss of nonetheless interesting findings.

The therapeutic potential of bullets could be assessed by estimating their ability at reducing the size of the pathological basins, namely the basins of the pathological attractors. This is a more permissive criterion since therapeutic bullets no longer have to necessarily remove the pathological attractors. Reducing the size of a pathological basin renders the corresponding pathological attractor less reachable, and then the associated pathological phenotype less likely. This new criterion includes the previous one since removing an attractor means reducing its basin to the empty set. Consequently, therapeutic bullets obtainable with the previous criterion are still obtainable.

1.3 Extending to multivalued logic

One of the main limitations of Boolean models is that variables can take only two values, which can be quite simplistic. Depending on what is modeled, such as activity level of enzymes or abundance of gene products, considering more than two levels can be more appropriate. Without leaving the logic-based modeling formalism, one solution is to extend Boolean logic to multivalued logic [22]. With multivalued logic, a finite number h of values in $[0; 1]$ is used, allowing variables to model more than two levels. For example, the level 0.5 can be introduced in order to model partial activations of enzymes, or moderate concentrations of gene products.

2 Methods

2.1 Additional definitions

In addition to the concepts defined in the previous article [1], and briefly recalled in *Appendix 5.1* page 13, here are some supplementary definitions:

- **physiological state space**: the state space S_{physio} of the physiological variant
- **pathological state space**: the state space S_{patho} of the pathological variant
- **testing state space**: the state space S_{test} of the pathological variant under the influence of a bullet
- **physiological basin**: the basin $B_{physio,i}$ of a physiological attractor $a_{physio,i}$
- **pathological basin**: the basin $B_{patho,i}$ of a pathological attractor $a_{patho,i}$
- n**-bullet**: a bullet made of n targets

2.2 Handling asynchronous updating

To implement asynchronous updating, the corresponding algorithms of BoolNet were adapted. BoolNet is an R[1] package for generation, reconstruction and analysis of Boolean networks [23]. Asynchronous updating is implemented so that only one randomly selected variable is updated at each iteration. This random selection is made according to a uniform distribution and implies that the network is no longer deterministic. To do so, given a Boolean network, BoolNet uses the three following functions:

- **AsynchronousAttractorSearch**: this function computes the attractor set of the supplied Boolean network by using the two following functions
- **ForwardSet**: this function computes the forward reachable set of a state and considers it as a candidate attractor
- **ValidateAttractor**: this function checks if a forward reachable set is a terminal strongly connected component (terminal SCC), that is an attractor

The forward reachable set $Fwd_{\boldsymbol{x}} \subset S$ of a state $\boldsymbol{x} \in S$ is the set made of the states reachable from $\boldsymbol{x}$, including $\boldsymbol{x}$ itself. A terminal SCC is a set $tSCC \subset S$ made of the forward reachable sets of its states: $\forall \boldsymbol{x} \in tSCC$, $Fwd_{\boldsymbol{x}} \subset tSCC$. As a consequence, when a terminal SCC is reached, the system can not escape it: this is an attractor in the sense of asynchronous Boolean networks [24]. Asynchronous Boolean networks with random updating are not deterministic: their attractors are no longer deterministic sequences of states, namely cycles, but terminal SCCs. To find an attractor, a long random walk is performed in order to reach an attractor with high probability. This candidate attractor is then validated, or not, by checking if it is a terminal SCC.

2.3 Managing basin sizes for therapeutic purposes

To implement the new criterion for selecting therapeutic bullets, kali considers a bullet as therapeutic if it increases $card \bigcup B_{physio,i}$ in S_{test} without creating *de*

[1] `https://www.r-project.org/`

novo attractors. Knowing that the attractors are either physiological or pathological, increasing $card \bigcup B_{physio,i}$ is equivalent to decreasing $card \bigcup B_{patho,i}$. The goal is to increase the physiological part of the pathological state space, or equivalently to decrease its pathological part. Consequently, a pathologically disturbed biological network receiving such a therapeutic bullet tends to, but not necessarily reaches, an overall physiological behavior. However, as with the previous criterion, it does not ensure that all the physiological attractors are preserved. *A fortiori*, it does not ensure that their basin remains unchanged. This means that a therapeutic bullet can also alter the reachability of the physiological attractors. Nevertheless, as with the previous criterion, this is a matter of choice between a therapeutic bullet or not.

The therapeutic potential of a bullet is expressed by its gain. It is displayed as follow: $x\% \rightarrow y\%$ where $card \bigcup B_{physio,i} = x\%$ of $card\ S_{patho}$ and $y\%$ of $card\ S_{test}$. Therefore, in order to increase the physiological part of the pathological state space, a therapeutic bullet has to make $y \geq x$. Note that $y = x$ is allowed. In this particular case, it is conceivable that several pathological basins changed in size without changing the size of their union. In other words, the composition of the pathological part changed while its size did not. This can be therapeutic if, for example, the basin of a weakly pathological attractor increased at the expense of the basin of a heavily pathological attractor.

The increase of the physiological part can be subjected to a threshold δ: $y \geq x$ becomes $y - x \geq \delta$. As x and y, δ is expressed in % of $card\ S_{patho/test}$. This threshold is introduced to allow the stringency of kali to be tuned. By the way, using this threshold also decreases the probability to obtain misassessed therapeutic bullets due to roundoff errors, or sampling errors when the state space is too big to be wholly computed.

A therapeutic bullet as defined by the previous criterion, namely which removes all the pathological attractors, makes *de facto* $card \bigcup B_{physio,i} = 100\%$ of $card\ S_{test}$. As already mentioned, the previous criterion is included in this new one: therapeutic bullets obtainable with the former are also obtainable with the latter.

2.4 Extending to multivalued logic

Multivalued logic requires suitable operators to be introduced. One solution is to use a mathematical formulation of the Boolean operators which also works with multivalued logic, just as the Zadeh operators. These operators are a generalization of the Boolean ones proposed for fuzzy logic by its pioneer Lotfi Zadeh [25]. Their mathematical formulation is:

$$\begin{aligned} x \wedge y &= min(x, y) \\ x \vee y &= max(x, y) \\ \neg x &= 1 - x \end{aligned}$$

With a h-valued logic, $card\ S = h^n$, which raises more computational difficulties than with Boolean logic. The same applies to the testable bullets since there are h^r possible modality arrangements and then $(n! \cdot h^r)/(r! \cdot (n - r)!)$ possible bullets, where r is the number of targets per bullet and n the number of nodes in the network. To illustrate how kali works with multivalued logic

without overloading it, a 3-valued logic is used where $\{0, 0.5, 1\}$ is the domain of value: $x_i \in \{0, 0.5, 1\}$. 0 and 1 have the same meaning as in Boolean logic while 0.5 is an intermediate truth degree which can be seen as an intermediate level of activity or abundance depending on what is modeled. By the way, $S = \{0, 0.5, 1\}^n$ and $moda_i \in \{0, 0.5, 1\}$.

2.5 Example network

To illustrate what results from the updates made on kali, a fictive network is used. This fictive network is specifically designed for illustration and is not intended to model a real biological phenomenon. This example network is depicted in *Figure 1* page 7. Among the three updates made on kali, only the asynchronous updating and the management of basin sizes are illustrated. Multivalued logic is illustrated in *Appendix 5.2* page 15.

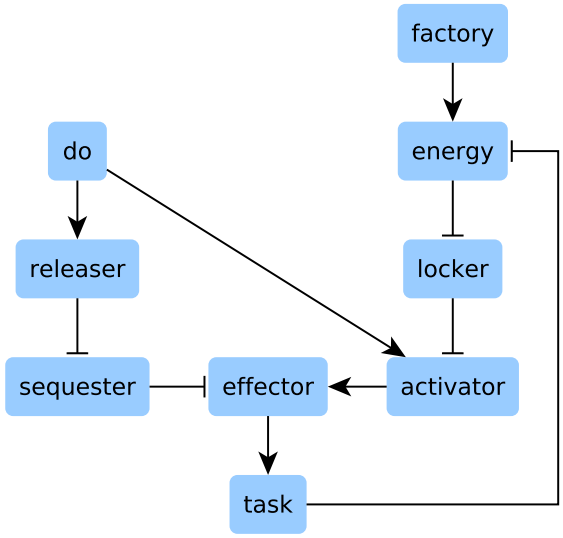

Figure 1: This network, running in a fictive cell, controls the execution of a task according to two inputs: i) the do instruction, which tells the task to be performed, and ii) energy supply. The task consumes energy and it must not be performed if no energy is available, even if the do instruction is sent. The task is initiated by an effector which is maintained inactive by a sequester. The do instruction activates a releaser which suppresses the sequestering activity of the sequester, thus releasing the effector. However, to initiate the task, in addition to be released the effector has also to be activated by an activator. When released and activated, the effector initiates the task. To ensure that the task is performed only if energy is available, a locker maintains the activator in an inactive state when there is no energy, even if the do instruction is there. Concerning the factory, it ensures that energy is supplied.

Below are the Boolean equations encoding the example network:

$$
\begin{aligned}
do &= do \\
factory &= factory \\
energy &= (energy \wedge \neg task) \vee factory \\
locker &= \neg energy \\
releaser &= do \\
sequester &= \neg releaser \\
activator &= do \wedge \neg locker \\
effector &= activator \wedge \neg sequester \\
task &= effector
\end{aligned}
$$

The do instruction and the factory are the two inputs: they are equal to themselves. The equation of energy tells that energy is present if the factory is active, even if the task is running: the factory has a sufficient capacity. However, if the factory is not active, energy disappears as soon as the task is initiated. Concerning the activator and the effector, their equations tell that their respective inhibitor takes precedence: whatever is the state of the other nodes, if the inhibitor is active then the target is not.

The physiological variant $\boldsymbol{f}_{physio}$ is the network as described above. The pathological variant $\boldsymbol{f}_{patho}$ is the network plus a constitutive inactivation of the locker: the execution of the task no longer considers if sufficient energy is available. Consequently, f_{locker} becomes $locker = 0$ in $\boldsymbol{f}_{patho}$.

2.6 Implementation, code availability and license

kali, together with the example network, is implemented in Go[1], tested with Go version go1.7.3 linux/amd64 under Arch Linux[2]. It is licensed under the GNU General Public License[3] and freely available on GitHub[4] at `https://github.com/arnaudporet/kali`. The core of kali in pseudocode can be found in *Appendix 5.3* page 18.

3 Results

3.1 Attractor sets

The example network is computed asynchronously over the whole state space using Boolean logic. The resulting attractors can be studied according to four variables: the do instruction, the factory, the locker and the task. Note that in the initial conditions, energy can be present without a running factory. In this case, if the do instruction is sent then the energy is consumed by the task but not remade by the factory. With the physiological variant, the locker is expected to stop the task. However, with the pathological variant, an abnormal behavior is expected since the locker is constitutively inactivated. Below are the computed attractors:

[1] `https://golang.org`
[2] `https://www.archlinux.org`
[3] `https://www.gnu.org/licenses/gpl.html`
[4] `https://github.com`

- A_{physio}:

attractor	basin (% of $card\ S_{physio}$)	do	$factory$	$energy$	$locker$	$task$
$a_{physio1}$	17.8%	0	0	0	1	0
$a_{physio2}$	7.2%	0	0	1	0	0
$a_{physio3}$	25%	0	1	1	0	0
$a_{physio4}$	25%	1	0	0	1	0
$a_{physio5}$	25%	1	1	1	0	1

- A_{patho}:

attractor	basin (% of $card\ S_{patho}$)	do	$factory$	$energy$	$locker$	$task$
a_{patho1}	18.4%	0	0	0	0	0
$a_{physio2}$	6.6%	0	0	1	0	0
$a_{physio3}$	25%	0	1	1	0	0
a_{patho2}	25%	1	0	0	0	1
$a_{physio5}$	25%	1	1	1	0	1

With the physiological variant, the behavior is as expected: the task runs only if the do instruction is sent and only if the factory is able to remade the consumed energy. With the pathological variant, two pathological phenotypes represented by a_{patho1} and a_{patho2} appear. a_{patho1} is pathological because the locker is inactive while there is no available energy. However, it is weakly pathological since the do instruction is not sent: an operational locker is not mandatory since there is no task to stop. In contrast, a_{patho2} is heavily pathological since an operational locker is required to stop the task in absence of energy supply. In the fictive cell bearing this example network, a_{patho2} could drain all the energy content and then bring the cell to thermodynamical death. Moreover, a_{patho2} should not be neglected since its basin occupies 25% of the pathological state space.

3.2 Therapeutic bullets

Bullets are assessed for their therapeutic potential on the pathological variant $\boldsymbol{f}_{patho}$ according to the new criterion. The goal is to decrease the size of the pathological basins $B_{patho,i}$. All the bullets made of one to two targets are computed with a threshold of 5%. This choice of the threshold value is quite arbitrary. It tells that if the physiological part $\bigcup B_{physio,i}$ of the pathological state space S_{patho} occupies x% of it, then to be therapeutic a bullet has to bring this value above $(x+5)$%. Therefore, the increases below this threshold are considered not significant by kali. Even the choice to use a threshold could be arbitrary, as discussed in the *Methods* section page 6.

Knowing that $card\ \bigcup B_{physio,i} = 56.6\%$ of $card\ S_{patho}$, with a threshold of 5% the $1,2$-bullets have to make $card\ \bigcup B_{physio,i} \geq (56.6+5)\% = 61.6\%$ of $card\ S_{test}$ to be considered therapeutic. Below are the returned therapeutic bullets:

- 1-therapeutic bullets:

bullet	gain			$B_{physio1}$	$B_{physio2}$	$B_{physio3}$	$B_{physio4}$	$B_{physio5}$	B_{patho1}	B_{patho2}
$do[0]$	56.6%	$\rightarrow$	64.4%	0%	14.4%	50%	0%	0%	35.5%	0%
$factory[1]$	56.6%	$\rightarrow$	100%	0%	0%	50%	0%	50%	0%	0%

- 2-therapeutic bullets:

bullet		gain			$B_{physio1}$	$B_{physio2}$	$B_{physio3}$	$B_{physio4}$	$B_{physio5}$	B_{patho1}	B_{patho2}
$do[0]$	$factory[1]$	56.6%	$\rightarrow$	100%	0%	0%	100%	0%	0%	0%	0%
$do[1]$	$factory[1]$	56.6%	$\rightarrow$	100%	0%	0%	0%	0%	100%	0%	0%
$do[0]$	$energy[1]$	56.6%	$\rightarrow$	100%	0%	50%	50%	0%	0%	0%	0%
$do[0]$	$locker[0]$	56.6%	$\rightarrow$	64.1%	0%	14.1%	50%	0%	0%	35.9%	0%
$do[0]$	$releaser[0]$	56.6%	$\rightarrow$	62.9%	0%	12.9%	50%	0%	0%	37.1%	0%
$do[0]$	$sequester[1]$	56.6%	$\rightarrow$	62.5%	0%	12.5%	50%	0%	0%	37.5%	0%
$do[0]$	$activator[0]$	56.6%	$\rightarrow$	64.8%	0%	14.8%	50%	0%	0%	35.2%	0%
$do[0]$	$effector[0]$	56.6%	$\rightarrow$	67.8%	0%	17.8%	50%	0%	0%	32.2%	0%
$do[0]$	$task[0]$	56.6%	$\rightarrow$	73.2%	0%	23.2%	50%	0%	0%	26.8%	0%
$factory[1]$	$energy[1]$	56.6%	$\rightarrow$	100%	0%	0%	50%	0%	50%	0%	0%
$factory[1]$	$locker[0]$	56.6%	$\rightarrow$	100%	0%	0%	50%	0%	50%	0%	0%

where $x[y]$ means that the variable x has to be set to the value y. For example, the bullet $do[0]$ $factory[1]$ tells that the do instruction has to be abolished while the factory has to be maintained active.

All the returned therapeutic bullets not removing all the pathological attractors exhibit the ability to suppress the basin of a_{patho2} while increasing the one of a_{patho1}. Certainly, removing all the pathological attractors should be better, but knowing the a_{patho2} is more pathological than a_{patho1}, such therapeutic bullets can nevertheless be interesting. With the previous criterion, namely removing all the pathological attractors, these therapeutic bullets are not obtainable, thus highlighting fewer therapeutic strategies.

Some of the found therapeutic bullets enable physiological attractors required by the pathological variant to react properly to the do instruction. For example, the bullet $factory[1]$ enables $a_{physio3}$ and $a_{physio5}$ which correspond respectively to "no do, no task" and "do the task, energy supply". However, the remaining of the therapeutic bullets, such as $do[0]$ $releaser[0]$ or $do[1]$ $factory[1]$, either disable or force the do instruction, thus either suppressing or forcing the task. A network which can not do the task or, at the opposite, which permanently does it, may not be therapeutically interesting even if energy is supplied.

None of the found therapeutic bullets suggest to reverse the constitutive inactivation of the locker by making it constitutively active. This highlight that applying the opposite action of the pathological disturbance is not necessarily a solution, which can appear counterintuitive. This might be due to the fact that biological entities subjected to pathological disturbances belong to complex networks, resulting in behaviors which can not be predicted by mind [26, 27]. This is where computational tools, and their growing computing capabilities, can help owing to their integrative power [28–32].

Also, none of the found therapeutic bullets allow the recovery of all the physiological attractors: there are no golden bullets. In a general manner, the components of a biological network should be able to take several states, such as enzymes which should be active when suitable. Consequently, healing a pathologically disturbed biological network by maintaining some of its components in a particular state should not allow the recovery of a complete and healthy behavior. This is a limitation of the method implemented in kali. This limitation is common in biomedicine while not necessarily being an issue. For example, statins are well known lipid-lowering drugs widely used in cardiovascular diseases with proven benefits [33, 34]. They inhibit an enzyme, the HMG-CoA reductase, and they do it constantly, just as the targets are modulated in the therapeutic bullets returned by kali. The HMG-CoA reductase is component of a complex metabolic network and maintaining it in an inhibited state should not allow this network to run properly, maybe causing some adverse effects. Nevertheless, such as with all drugs, this is a matter of benefit-risk ratio. All of this is to say that there are no perfect method for counteracting diseases and that computational tools, such as kali, can help scientists but can certainly not replace their expertise. Human expertise is mandatory to assess the concrete meaning and usability of the results, and ultimately to take decisions.

4 Conclusion

kali can now work on asynchronous Boolean networks, in addition to synchronous ones. This is probably the most important update which had to be done. Indeed, the asynchronous updating is frequently used by biomodelers since it is supposed to be more realistic, as discussed in the *Introduction* section

page 3. Therefore, a computational tool aimed at working on models built by the scientific community, such as kali, has to handle this updating scheme. It should be noted that there are more than one asynchronous updating scheme. The one implemented in kali is the most popular and is named the general asynchronous updating: one randomly selected variable is updated at each iteration. However, other asynchronous updating methods exist. For example, with the random order updating, all the variables are updated at each iteration but in a randomly built order. Implementing various asynchronous updating schemes in kali may be a required future improvement.

kali now uses a new criterion for selecting therapeutic bullets which brings a wider range of targeting strategies intended to push pathological behaviors toward physiological ones. This new criterion is based on a more permissive assumption stating that reducing the reachability of pathological attractors is therapeutic. For an *in silico* tool, such as kali, being a little bit more permissive may be important since theoretical findings have to outlive the bottleneck separating prediction to reality. With a too strict assessment of therapeutic bullets, the risk of highlighting too few candidate targets, or to miss some interesting ones, could be hight. Moreover, predicted does not necessarily mean true: an *in silico* prediction of apparently poor interest can reveal itself of great interest, and *vice versa*.

This new criterion also brings a finer assessment of therapeutic bullets since all the percentages between $card \bigcup B_{physio,i}$ in S_{patho} and 100% in S_{test} are considered. With the previous criterion, the only therapeutic potential was $card \bigcup B_{physio,i} = 100\%$ in S_{test}, thus reducing the assessment to therapeutic or not. However, things are not necessarily so dichotomous but rather nuanced, so the assessment of therapeutic bullets should be nuanced too.

kali can now compute with multivalued logic. Allowing variables to take an arbitrary finite number of values should enable to more accurately model biological processes and produce more fine-tuned therapeutic bullets. However, this accuracy and fine-tuning are at the cost of an increased computational requirement. Indeed, the cardinality of the state space depends on the size of the model and the used logic. Therefore, the size of the model and the used logic should be balanced: the smaller the model is, the more variables should be finely valued. For example, for an accurate therapeutic investigation, the model should only contain the essential and specific pieces of the studied pathophysiology, modeled by a finely valued logic. On the other hand, for a broad therapeutic investigation, a more exhaustive model can be used but modeled by a coarse-grained logic. Finally, it should be noted that the ultimate multivalued logic is the infinitely valued one, which is fuzzy logic [35]. With fuzzy logic, the whole $[0; 1]$ is used to valuate variables, which might bring the best accuracy for the qualitative modeling formalism [36–38].

Two additional improvements are envisioned for kali. The first one is to allow *de novo* attractors to appear in A_{test}. For example, it is conceivable that a bullet greatly decreases the pathological basins while creating a new attractor not belonging to A_{physio} nor to A_{patho}. Such a *de novo* attractor might be defined as not physiological, and then pathological. However, if it is weakly pathological and induced by a bullet which greatly decreases the basin of other, and heavier, pathological attractors, such a case should be allowed to be investigated.

The second improvement is to allow partial matching when checking if an

attractor is associated with a physiological phenotype by comparing it to the physiological attractors. Currently, an attractor which does not match a physiological attractor is considered pathologic. However, it is conceivable that some variables not exhibiting a physiological behavior in an attractor do not pathologically impact the associated phenotype. To allow such a case to be considered, some variables within attractors should be allowed to not be matched when assessing the associated phenotype. This suggests the concept of decisive variables. Decisive variables would be variables whose the behavior in the attractors is sufficient to biologically interpret the associated phenotype. Therefore, kali could allow non-decisive variables to not be matched. Ultimately, this could allow the modeler to specify himself what a physiological attractor is without computing a physiological variant.

5 Appendices

5.1 Appendix 1: recall of previous concepts

5.1.1 Biological networks

A network is a digraph $G = (V, E)$ where $V = \{v_1, \ldots, v_n\}$ is the set containing the nodes and $E = \{(v_{i,1}, v_{j,1}), \ldots, (v_{i,m}, v_{j,m})\} \subset V^2$ the set containing the edges. In practice, nodes represent entities and edges represent binary relations $R \subset V^2$ involving them: $v_i \ R \ v_j$ [39]. It indicates that the node v_i exerts an influence on the node v_j. For example, in gene regulatory networks [40], v_i can be a transcription factor while v_j a gene product. The edges are frequently signed so that they indicate if v_i exerts a positive or a negative influence on v_j, such as inhibitions or activations.

5.1.2 Boolean networks

A Boolean network is a network where nodes are Boolean variables x_i and edges (x_i, x_j) the *is input of* relation: x_i *is input of* x_j. Each x_i has $b_i \in [\![0, n]\!]$ inputs. Depending on the updating scheme, at each iteration $k \in [\![k_0, k_{end}]\!]$, one or more x_i are updated using their associated Boolean function f_i and their inputs, as in the following pseudocode representing a synchronous updating:

for $k \leftarrow k_0, \ldots, k_{end}$
  $x_1 \leftarrow f_1(x_{1,1}, \ldots, x_{1,b_1})$
  $\vdots$
  $x_n \leftarrow f_n(x_{n,1}, \ldots, x_{n,b_n})$
end for

which can be written in a more concise form:

for $k \leftarrow k_0, \ldots, k_{end}$
  $\boldsymbol{x} \leftarrow \boldsymbol{f}(\boldsymbol{x})$
end for

where $\boldsymbol{f} = (f_1, \ldots, f_n)$ is the Boolean transition function and $\boldsymbol{x} = (x_1, \ldots, x_n)$ the state vector. The value of $\boldsymbol{x}$ belongs to the state space $S = \{0, 1\}^n$ which is the set containing the possible states.

The set $A = \{a_1, \ldots, a_p\}$ containing the attractors is the attractor set. An attractor a_i is a collection of states $(\boldsymbol{x}_1, \ldots, \boldsymbol{x}_q)$ such that once the system

reaches a state $\boldsymbol{x}_j \in a_i$, it can subsequently visit the states of a_i and no other ones: the system can not escape. The set $B_i \subset S$ containing the $\boldsymbol{x} \in S$ from which a_i can be reached is its basin of attraction, or simply basin.

5.1.3 Definitions

- **physiological phenotype**: A phenotype which does not impair the life quantity /quality of the organism which exhibits it.
- **pathological phenotype**: A phenotype which impairs the life quantity /quality of the organism which exhibits it.
- **variant (of a biological network)**: Given a biological network, a variant is one of its versions, namely the network plus eventually some modifications.
- **physiological variant**: A variant which produces only physiological phenotypes. This is the biological network as it should be, namely the one of healthy organisms.
- **pathological variant**: A variant which produces at least one pathological phenotype, or which fails to produce at least one physiological phenotype. This is a dysfunctional version of the biological network, namely a version found in ill organisms.
- **physiological attractor set**: The attractor set A_{physio} of the physiological variant.
- **pathological attractor set**: The attractor set A_{patho} of the pathological variant.
- **physiological Boolean transition function**: The Boolean transition function $\boldsymbol{f}_{physio}$ of the physiological variant.
- **pathological Boolean transition function**: The Boolean transition function $\boldsymbol{f}_{patho}$ of the pathological variant.
- **physiological attractor**: An attractor a_i such that $a_i \in A_{physio}$. Note that it does not exclude the possibility that $a_i \in A_{patho}$ in addition to $a_i \in A_{physio}$.
- **pathological attractor**: An attractor a_i such that $a_i \notin A_{physio}$.
- **modality**: The perturbation $moda_i \in \{0, 1\}$ applied on a node v_j of the network, either activating ($moda_i = 1$) or inactivating ($moda_i = 0$). At each iteration, $moda_i$ overwrites $f_j(\boldsymbol{x})$ making $x_j = moda_i$.
- **target**: A node $targ_i$ of the network on which a $moda_i$ is applied.
- **bullet**: A couple (c_{targ}, c_{moda}) where $c_{targ} = (targ_1, \ldots, targ_r)$ is a combination without repetition of targets and $c_{moda} = (moda_1, \ldots, moda_r)$ an arrangement with repetition of modalities. $moda_i$ is intended to be applied on $targ_i$.

5.2 Appendix 2: multivalued case

Below is the multivalued version of the example network:

$$\begin{aligned} do &= do \\ factory &= factory \\ energy &= max(min(energy, 1 - task), factory) \\ locker &= 1 - energy \\ releaser &= do \\ sequester &= 1 - releaser \\ activator &= min(do, 1 - locker) \\ effector &= min(activator, 1 - sequester) \\ task &= effector \end{aligned}$$

where the Boolean operators are replaced by the Zadeh ones. To take advantage of multivalued logic, f_{locker} becomes $locker = min(1 - energy, 0.5)$ in $\boldsymbol{f}_{patho}$. This equation tells that the locker is actionable when required, namely when there is no energy, but that it is unable at being fully operational due to some pathological defects: the maximal value of f_{locker} in $\boldsymbol{f}_{patho}$ is 0.5. As mentioned in the article, 0.5 can be interpreted as an incomplete activation, or an incomplete inhibition depending on what is modeled. Consequently, the activator is at most partly inhibited by the locker when no energy is available, allowing the task to be nevertheless performed. However, the task itself will be moderately performed.

5.2.1 Attractor sets

Below are the computed attractors:

- A_{physio}:

attractor	basin (% of $card\ S_{physio}$)	do	$factory$	$energy$	$locker$	$task$
$a_{physio1}$	6.1%	0	0	0	1	0
$a_{physio2}$	4.5%	0	0	0.5	0.5	0
$a_{physio3}$	2.5%	0	0	1	0	0
$a_{physio4}$	9.7%	0	0.5	0.5	0.5	0
$a_{physio5}$	1.8%	0	0.5	1	0	0
$a_{physio6}$	10.8%	0	1	1	0	0
$a_{physio7}$	6.5%	0.5	0	0	1	0
$a_{physio8}$	4.8%	0.5	0	0.5	0.5	0.5
$a_{physio9}$	10.3%	0.5	0.5	0.5	0.5	0.5
$a_{physio10}$	10.6%	0.5	1	1	0	0.5
$a_{physio11}$	7.3%	1	0	0	1	0
$a_{physio12}$	3.2%	1	0	0.5	0.5	0.5
$a_{physio13}$	10.3%	1	0.5	0.5	0.5	0.5
$a_{physio14}$	11.6%	1	1	1	0	1

- A_{patho}:

attractor	basin (% of $card\ S_{patho}$)	do	$factory$	$energy$	$locker$	$task$
a_{patho1}	6.2%	0	0	0	0.5	0
$a_{physio2}$	4.7%	0	0	0.5	0.5	0
$a_{physio3}$	2.2%	0	0	1	0	0
$a_{physio4}$	9.7%	0	0.5	0.5	0.5	0
$a_{physio5}$	1.8%	0	0.5	1	0	0
$a_{physio6}$	10.8%	0	1	1	0	0
a_{patho2}	5.5%	0.5	0	0	0.5	0.5
$a_{physio8}$	5.8%	0.5	0	0.5	0.5	0.5
$a_{physio9}$	10.3%	0.5	0.5	0.5	0.5	0.5
$a_{physio10}$	10.6%	0.5	1	1	0	0.5
a_{patho3}	7.3%	1	0	0	0.5	0.5
$a_{physio12}$	3.2%	1	0	0.5	0.5	0.5
$a_{physio13}$	10.3%	1	0.5	0.5	0.5	0.5
$a_{physio14}$	11.6%	1	1	1	0	1

$a_{physio1}$, $a_{physio3}$, $a_{physio6}$, $a_{physio11}$ and $a_{physio14}$ are the physiological attractors found in the Boolean case, with a different numbering due to additional attractors coming from multivalued logic. Indeed, given that $\{0,1\} \subset \{0,0.5,1\}$ and that the Zadeh operators also work with Boolean logic, the Boolean results are still obtainable. The same does not apply to the pathological attractors because f_{locker} in $\boldsymbol{f}_{patho}$ differs between the Boolean and multivalued case.

For example, $a_{physio13}$ indicates that the do instruction is sent while energy is partly supplied. Consequently, the locker is partly activated resulting in a partial inhibition of the activator. The task is thus moderately performed despite full do instruction. Concerning the pathological attractors, as an example, a_{patho3} indicates that the do instruction is sent in total absence of energy supply. Consequently, the locker should be fully activated to prevent the task to be performed. However, due to some pathological defects, it is at most partly activated. Therefore, the task is performed in total absence of energy. However, since the locker is partly operational, the task is not performed at its maximum rate.

Among the pathological attractors, a_{patho1} can be considered weakly pathological. In a_{patho1}, the locker should be fully activated since there is no energy. However, there is no do instruction and therefore no task to stop. On the other hand, a_{patho2} and a_{patho3} are more pathological since the task is performed while no energy is available.

5.2.2 Therapeutic bullets

Below are the returned therapeutic bullets:

- 1-therapeutic bullets:

bullet	gain	$B_{physio1}$ $B_{physio10}$	$B_{physio2}$ $B_{physio11}$	$B_{physio3}$ $B_{physio12}$	$B_{physio4}$ $B_{physio13}$	$B_{physio5}$ $B_{physio14}$	$B_{physio6}$ B_{patho1}	$B_{physio7}$ B_{patho2}	$B_{physio8}$ B_{patho3}	$B_{physio9}$
$factory$[0.5]	81% → 100%	0%	0%	0%	29.3%	6.1%	0%	0%	0%	32.2%
		0%	0%	0%	32.4%	0%	0%	0%	0%	
$factory$[1]	81% → 100%	0%	0%	0%	0%	0%	35.4%	0%	0%	0%
		32.2%	0%	0%	0%	32.4%	0%	0%	0%	

- 2-therapeutic bullets:

bullet		gain	$B_{physio1}$ $B_{physio10}$	$B_{physio2}$ $B_{physio11}$	$B_{physio3}$ $B_{physio12}$	$B_{physio4}$ $B_{physio13}$	$B_{physio5}$ $B_{physio14}$	$B_{physio6}$ B_{patho1}	$B_{physio7}$ B_{patho2}	$B_{physio8}$ B_{patho3}	$B_{physio9}$
do[0]	$factory$[0.5]	81% → 100%	0%	0%	0%	84%	16%	0%	0%	0%	0%
			0%	0%	0%	0%	0%	0%	0%	0%	
do[0]	$factory$[1]	81% → 100%	0%	0%	0%	0%	0%	100%	0%	0%	0%
			0%	0%	0%	0%	0%	0%	0%	0%	
do[0.5]	$factory$[0.5]	81% → 100%	0%	0%	0%	0%	0%	0%	0%	0%	100%
			0%	0%	0%	0%	0%	0%	0%	0%	
do[0.5]	$factory$[1]	81% → 100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
			100%	0%	0%	0%	0%	0%	0%	0%	
do[1]	$factory$[0.5]	81% → 100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
			0%	0%	0%	100%	0%	0%	0%	0%	
do[1]	$factory$[1]	81% → 100%	0%	0%	0%	0%	0%	0%	0%	0%	0%
			0%	0%	0%	0%	100%	0%	0%	0%	
do[0]	$energy$[1]	81% → 100%	0%	0%	34.9%	0%	32.1%	33%	0%	0%	0%
			0%	0%	0%	0%	0%	0%	0%	0%	
do[0]	$task$[0]	81% → 89%	0%	11.6%	12.3%	21.3%	10.8%	33%	0%	0%	0%
			0%	0%	0%	0%	0%	11%	0%	0%	
do[0.5]	$task$[0.5]	81% → 89.4%	0%	0%	0%	0%	0%	0%	0%	24.3%	32.1%
			33%	0%	0%	0%	0%	0%	10.6%	0%	
$factory$[0]	$energy$[0.5]	81% → 100%	0%	35.4%	0%	0%	0%	0%	0%	32.2%	0%
			0%	0%	32.4%	0%	0%	0%	0%	0%	
$factory$[0.5]	$energy$[0.5]	81% → 100%	0%	0%	0%	35.4%	0%	0%	0%	0%	32.2%
			0%	0%	0%	32.4%	0%	0%	0%	0%	
$factory$[1]	$energy$[1]	81% → 100%	0%	0%	0%	0%	0%	35.4%	0%	0%	0%
			32.2%	0%	0%	0%	32.4%	0%	0%	0%	
$factory$[1]	$locker$[0]	81% → 100%	0%	0%	0%	0%	0%	35.4%	0%	0%	0%
			32.2%	0%	0%	0%	32.4%	0%	0%	0%	

For example, the therapeutic bullet $factory[1]$ $locker[0]$ may be interesting. It suppresses all the pathological attractors while maintaining three physiological attractors allowing the network to properly respond to the three possible levels of the do instruction. Moreover, the basins of these three physiological attractors, namely $a_{physio6}$, $a_{physio10}$ and $a_{physio14}$, equally span the state space, making them equally reachable. On the other hand, the therapeutic bullet $do[0.5]$ $factory[0.5]$ seems to be less interesting. While this bullet also suppresses all the pathological attractors, it enables only one physiological attractor. In this physiological attractor, namely $a_{physio9}$, all the variables are at their intermediate level. Consequently, the network can not fulfill its switching function.

5.3 Appendix 3: core of kali

Below is the core of kali in pseudocode derived from its Go[1] sources, freely available on GitHub[2] at `https://github.com/arnaudporet/kali` under the GNU General Public License[3].

5.3.1 Defined types

structure *Attractor*// an attractor
  field $Name$// its name, either a_{physio} or a_{patho}
  field $Basin$// the size of its basin
  field $States$// its states
end structure

structure *Bullet*// a bullet
  field $Targ$// its target combination, namely c_{targ}
  field $Moda$// its modality arrangement, namely c_{moda}
  field $Gain$// its gain
  field $Cover$// the size of each basin under its influence
end structure

5.3.2 Parameters

nodes// the node names, ex: $(PI3K, Akt, mTOR, \ldots)$
Ω// the domain of the used logic, ex: $\{0, 0.5, 1\}$ for three-valued logic
$sync$// use synchronous updating (1) or not (0)
$whole$// compute the whole state space (1) or not (0)
max_S// the maximal size of the sample of the state space when $whole = 0$
max_k// the number of iterations for a random walk (asynchronous only)
n_{targ}// the number of targets per bullet
max_{targ}// the maximum number of target combinations to test
max_{moda}// the maximum number of modality arrangements to test
δ// the threshold for a bullet to be considered therapeutic

[1] `https://golang.org`
[2] `https://github.com`
[3] `https://www.gnu.org/licenses/gpl.html`

5.3.3 Functions

function $DoTheJob(\boldsymbol{f}_{physio}, \boldsymbol{f}_{patho}, n_{targ}, max_{targ}, max_{moda}, max_S, max_k, \delta,$
$sync, \boldsymbol{nodes}, \Omega, whole)$
// do the job, this is the main function
$n \leftarrow Size(\boldsymbol{nodes})$// dimension of the state space S
select *whole*
case 0// use a sample of S
$S \leftarrow GenArrangs(\Omega, n, max_S)$
case 1// use all S
$S \leftarrow GenSpace(\Omega, n)$
end select
$A_{physio} \leftarrow ComputeAttractorSet(\boldsymbol{f}_{physio}, S, \varnothing, max_k, 0, sync, \emptyset)$
$A_{patho} \leftarrow ComputeAttractorSet(\boldsymbol{f}_{patho}, S, \varnothing, max_k, 1, sync, A_{physio})$
$A_{versus} \leftarrow GetVersus(A_{patho})$
$C_{targ} \leftarrow GenCombis(\{1, \ldots, n\}, n_{targ}, max_{targ})$// target combinations
$C_{moda} \leftarrow GenArrangs(\Omega, n_{targ}, max_{moda})$// modality arrangements
if $A_{versus} \neq \emptyset$
$B_{therap} \leftarrow ComputeTherapeuticBullets(\boldsymbol{f}_{patho}, S, C_{targ}, C_{moda}, max_k,$
$\delta, sync, A_{physio}, A_{patho}, A_{versus})$// therapeutic bullets
end if
return $S, A_{physio}, A_{patho}, A_{versus}, C_{targ}, C_{moda}, B_{therap}$
end function

$Size(container)$ returns the number of items in *container*.

$GenSpace(\Omega, n)$ returns the state space of the vectors made of n values from Ω.

$GenArrangs(\Omega, n, max_{arrang})$ returns max_{arrang} arrangements with repetition made of n elements from Ω. If max_{arrang} exceeds its maximal possible value then it is automatically decreased to its maximal possible value.

$GenCombis(\Omega, n, max_{combi})$ returns max_{combi} combinations without repetition made of n elements from Ω. If max_{combi} exceeds its maximal possible value then it is automatically decreased to its maximal possible value.

A_{physio} is computed without bullet ($b \leftarrow \varnothing$), without reference set ($A_{ref} \leftarrow \emptyset$) and with physiological setting ($setting \leftarrow 0$).

A_{patho} is computed without bullet ($b \leftarrow \varnothing$), with reference set ($A_{ref} \leftarrow A_{physio}$) and with pathological setting ($setting \leftarrow 1$).

A_{versus} is not a true attractor set but the set containing the pathological attractors: $A_{versus} \subset A_{patho}$. A_{patho} can contains physiological attractors if the pathological variant exhibits some of them. However, A_{versus} only contains the pathological attractors.

Therapeutic bullets are computed only if there are pathological basins to shrink, namely only if $A_{versus} \neq \emptyset$.

```
function f_physio(x)
    // the transition function of the physiological variant
    y[1] ← f_physio[1](x)// update x_1 with f_physio,1
    ⋮
    y[n] ← f_physio[n](x)// update x_n with f_physio,n
    return y
end function

function f_patho(x)
    // the transition function of the pathological variant
    y[1] ← f_patho[1](x)// update x_1 with f_patho,1
    ⋮
    y[n] ← f_patho[n](x)// update x_n with f_patho,n
    return y
end function

function ComputeAttractor(f, x_0, b, max_k, sync)
    // compute the attractor a reachable from x_0
    select sync
        case 1// search a cycle
            a.States ← ReachCycle(f, x_0, b)
        case 0// search a terminal SCC
            for
                a.States ← GoForward(f, Walk(f, x_0, b, max_k), b)// candidate
                if IsTerminal(a, f, b)// candidate is a terminal SCC
                    break// then it is an asynchronous attractor
                end if
            end for
    end select
    return a
end function

function ComputeAttractorSet(f, S, b, max_k, setting, sync, A_ref)
    // compute an attractor set A, namely A_physio, A_patho or A_test
    A ← {}
    select setting// select the appropriate default attractor name
        case 0
            name ← a_physio
        case 1
            name ← a_patho
    end select
    for i ← 1, ..., Size(S)// browse S
        a ← ComputeAttractor(f, S[i], b, max_k, sync)
        if ∃i_A : A[i_A] = a// a is already found
            A[i_A].Basin ← A[i_A].Basin + 1// then increase its basin
        else// new attractor
            a.Basin ← 1// then begin its basin
            A ← A ∪ {a}// and add it to the set
```

```
        end if
    end for
    for i ← 1, ..., Size(A) // browse A
        A[i].Basin ← 100 · A[i].Basin/Size(S) // translate to % of card S
    end for
    return SetNames(A, name, A_ref)
end function

function ComputeTherapeuticBullets(f_patho, S, C_targ, C_moda, max_k, δ, sync,
    A_physio, A_patho, A_versus)
    // compute a set B_therap of therapeutic bullets
    B_therap ← {}
    b.Gain[1] ← Sum(GetCover(A_physio, A_patho))
    for i_1 ← 1, ..., Size(C_targ) // browse target combinations to test
        for i_2 ← 1, ..., Size(C_Moda) // browse modality arrangements to test
            b.Targ ← C_targ[i_1] // the target combination to test
            b.Moda ← C_Moda[i_2] // the modality arrangement to test
            A_test ← ComputeAttractorSet(f_patho, S, b, max_k, 1, sync, A_physio)
            b.Gain[2] ← Sum(GetCover(A_physio, A_test))
            if IsTherapeutic(b, A_test, A_versus, δ) // b is therapeutic
                b.Cover ← GetCover(A_physio ∪ A_versus, A_test)
                B_therap ← B_therap ∪ {b} // then add it to the set
            end if
        end for
    end for
    return B_therap
end function
```

$Sum(container)$ returns the sum of the items of *container*.

$b.Gain$ is a couple $(gain1, gain2)$ where:

- $gain1$ is $card\ \bigcup B_{physio,i}$ in S_{patho}
- $gain2$ is $card\ \bigcup B_{physio,i}$ in S_{test}

expressed in % of $card\ S_{patho}$ and % of $card\ S_{test}$ respectively.

$b.Cover$ stores the size of the physiological and pathological basins in the testing state space.

```
function GetCover(A_1, A_2)
    // get the size of the B_1,i in S_2, in % of card S_2
    cover ← ()
    for i ← 1, ..., Size(A_1) // browse the attractors of A_1
        if ∃i_2 : A_2[i_2] = A_1[i] // A_1[i] also belongs to A_2
            cover ← Append(cover, A_2[i_2].Basin) // then get card B_1,i in S_2
        else // A_1[i] not in A_2
            cover ← Append(cover, 0) // then B_1,i is empty in S_2
        end if
    end for
```

```
    return cover
end function
```

$Append(container, item)$ returns *container* with *item* added to it.

```
function GetVersus(A_patho)
    // get the pathological attractors
    A_versus ← {}
    for i ← 1, ..., Size(A_patho) // browse the attractors of A_patho
        if IsSubString(A_patho[i].Name, patho) // not a physiological attractor
            A_versus ← A_versus ∪ {A_patho[i]} // then add it to the set
        end if
    end for
    return A_versus
end function
```

$IsSubString(s_1, s_2)$ checks if s_2 is a substring of s_1.

```
function GoForward(f, x_0, b)
    // compute the forward reachable set fwd of x_0 (asynchronous only)
    fwd ← {x_0} // fwd contains x_0 itself
    stack ← (x_0) // the stack of the visited states
    for
        x ← stack[Size(stack)] // get the last stack element
        stack ← stack[1, ..., Size(stack) − 1] // remove the last stack element
        y ← f(x) // prepare all the updated x_i
        for i ← 1, ..., Size(y) // browse the updated x_i
            z ← x // copy x to preserve its original value
            z[i] ← y[i] // update only one x_i
            z ← Shoot(z, b) // apply the bullet
            if z ∉ fwd // new state
                fwd ← fwd ∪ {z} // then add it to the set
                stack ← Append(stack, z) // and store it into the stack
            end if
        end for
        if Size(stack) = 0 // no new states to visit
            break // so the complete fwd is obtained
        end if
    end for
    return fwd
end function
```

```
function IsTerminal(a, f, b)
    // check if a candidate attractor is a terminal SCC (asynchronous only)
    for i ← 1, ..., Size(a.States) // browse the states of a
        if GoForward(f, a.States[i], b) ≠ a.States // fwd_i ∉ a
            return false // then not a terminal SCC
        end if
    end for
    return true // assumed to be a terminal SCC until proven otherwise
```

end function

function $IsTherapeutic(b, A_{test}, A_{versus}, \delta)$
  // check if a bullet is therapeutic
  if $b.Gain[2] - b.Gain[1] \geq \delta$// maybe therapeutic
    for $i \leftarrow 1, \ldots, Size(A_{test})$// browse the attractors of A_{test}
      if $IsSubString(A_{test}[i].Name, patho) \wedge A_{test}[i] \notin A_{versus}$
        return $false$// because of a *de novo* attractor
      end if
    end for
    return $true$// assumed to be therapeutic until proven otherwise
  else
    return $false$// below the threshold
  end if
end function

function $ReachCycle(\boldsymbol{f}, \boldsymbol{x}_0, b)$
  // compute the cycle reachable from $\boldsymbol{x}_0$ (synchronous only)
  $\boldsymbol{cycle} \leftarrow (\boldsymbol{x}_0)$// begin the trajectory
  $\boldsymbol{x} \leftarrow \boldsymbol{x}_0$
  for
    $\boldsymbol{x} \leftarrow Shoot(\boldsymbol{f}(\boldsymbol{x}), b)$// update and apply the bullet
    if $\exists i : \boldsymbol{cycle}[i] = \boldsymbol{x}$// cycle found in the trajectory
      $\boldsymbol{cycle} \leftarrow \boldsymbol{cycle}[i, \ldots, Size(\boldsymbol{cycle})]$// extract the cycle
      break// mission completed
    else// cycle not yet reached
      $\boldsymbol{cycle} \leftarrow Append(\boldsymbol{cycle}, \boldsymbol{x})$// then pursue the trajectory
    end if
  end for
  return $\boldsymbol{cycle}$
end function

function $SetNames(A, name, A_{ref})$
  // name the attractors of A according to A_{ref} (defaults to $name$)
  $y \leftarrow A$// copy A to return a copy
  $k \leftarrow 1$// initiate the default name numbering
  for $i \leftarrow 1, \ldots, Size(A)$// browse the attractors of A
    if $\exists i_{ref} : A_{ref}[i_{ref}] = A[i]$// $A[i]$ found in A_{ref}
      $y[i].Name \leftarrow A_{ref}[i_{ref}].Name$// then get its name in A_{ref}
    else// $A[i]$ not in A_{ref}
      $y[i].Name \leftarrow CatStrings(name, ToString(k))$// then default
      $k \leftarrow k + 1$// and increment the default name numbering
    end if
  end for
  return y
end function

$CatStrings(s_1, s_2)$ returns the concatenation of s_1 and s_2.

$ToString(item)$ returns the string corresponding to $item$.

This function names the attractors of A according to a reference set A_{ref}. If an attractor of A also belongs to A_{ref} then its name in A_{ref} is used, otherwise the default name, numbered with k, is used.

function $Shoot(\boldsymbol{x}, b)$
  // apply a bullet on a state
  $\boldsymbol{y} \leftarrow \boldsymbol{x}$// copy $\boldsymbol{x}$ to return a copy
  for $i \leftarrow 1, \ldots, Size(b.Targ)$// browse c_{targ}
    $\boldsymbol{y}[b.Targ[i]] \leftarrow b.Moda[i]$// apply $moda_i$ on $targ_i$
  end for
  return $\boldsymbol{y}$
end function

function $Walk(\boldsymbol{f}, \boldsymbol{x}_0, b, max_k)$
  // perform a random walk from $\boldsymbol{x}_0$ (asynchronous only)
  $\boldsymbol{x} \leftarrow \boldsymbol{x}_0$// start the trajectory
  for $k \leftarrow 1, \ldots, max_k$// until max_k
    $\boldsymbol{y} \leftarrow \boldsymbol{f}(\boldsymbol{x})$// prepare all the updated x_i
    $i \leftarrow RandInt(1, Size(\boldsymbol{x}))$// randomly choose one x_i
    $\boldsymbol{x}[i] \leftarrow \boldsymbol{y}[i]$// then update only the chosen x_i
    $\boldsymbol{x} \leftarrow Shoot(\boldsymbol{x}, b)$// and apply the bullet
  end for
  return $\boldsymbol{x}$
end function

$RandInt(a, b)$ returns a randomly selected integer between a and b according to a uniform distribution.

References

[1] Arnaud Poret and Jean-Pierre Boissel. An in silico target identification using boolean network attractors: avoiding pathological phenotypes. *Comptes rendus biologies*, 337(12):661–678, 2014.

[2] Nicolas Le Novere. Quantitative and logic modelling of molecular and gene networks. *Nature Reviews Genetics*, 16(3):146–158, 2015.

[3] Michelle L Wynn, Nikita Consul, Sofia D Merajver, and Santiago Schnell. Logic-based models in systems biology: a predictive and parameter-free network analysis method. *Integrative biology*, 4(11):1323–1337, 2012.

[4] Melody K Morris, Julio Saez-Rodriguez, Peter K Sorger, and Douglas A Lauffenburger. Logic-based models for the analysis of cell signaling networks. *Biochemistry*, 49(15):3216–3224, 2010.

[5] Reka Albert and Juilee Thakar. Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 6(5):353–369, 2014.

[6] Rui-Sheng Wang, Assieh Saadatpour, and Reka Albert. Boolean modeling in systems biology: an overview of methodology and applications. *Physical biology*, 9(5):055001, 2012.

[7] Johannes Jaeger and Nick Monk. Bioattractors: dynamical systems theory and the evolution of regulatory processes. *The Journal of physiology*, 592(11):2267–2281, 2014.

[8] Sung-Hwan Cho, Sang-Min Park, Ho-Sung Lee, Hwang-Yeol Lee, and Kwang-Hyun Cho. Attractor landscape analysis of colorectal tumorigenesis and its reversion. *BMC Systems Biology*, 10(1):96, 2016.

[9] Xiao Gan and Reka Albert. Analysis of a dynamic model of guard cell signaling reveals the stability of signal propagation. *BMC Systems Biology*, 10(1):78, 2016.

[10] Jose Davila-Velderrain, Juan C Martinez-Garcia, and Elena R Alvarez-Buylla. Modeling the epigenetic attractors landscape: toward a post-genomic mechanistic understanding of development. *Frontiers in genetics*, 6:160, 2015.

[11] Isaac Crespo, Thanneer M Perumal, Wiktor Jurkowski, and Antonio Del Sol. Detecting cellular reprogramming determinants by differential stability analysis of gene regulatory networks. *BMC systems biology*, 7(1):1, 2013.

[12] Herman F Fumia and Marcelo L Martins. Boolean network model for cancer pathways: predicting carcinogenesis and targeted therapy outcomes. *PloS one*, 8(7):e69008, 2013.

[13] Wei-Yi Cheng, Tai-Hsien Ou Yang, and Dimitris Anastassiou. Biomolecular events in cancer revealed by attractor metagenes. *PLoS Comput Biol*, 9(2):e1002920, 2013.

[14] Pau Creixell, Erwin M Schoof, Janine T Erler, and Rune Linding. Navigating cancer network attractors for tumor-specific therapy. *Nature biotechnology*, 30(9):842–848, 2012.

[15] MK Morris, DC Clarke, LC Osimiri, and DA Lauffenburger. Systematic analysis of quantitative logic model ensembles predicts drug combination effects on cell signaling networks. *CPT: Pharmacometrics & Systems Pharmacology*, 2016.

[16] Hyunho Chu, Daewon Lee, and Kwang-Hyun Cho. Precritical state transition dynamics in the attractor landscape of a molecular interaction network underlying colorectal tumorigenesis. *PloS one*, 10(10):e0140172, 2015.

[17] Abhishek Garg, Alessandro Di Cara, Ioannis Xenarios, Luis Mendoza, and Giovanni De Micheli. Synchronous versus asynchronous modeling of gene regulatory networks. *Bioinformatics*, 24(17):1917–1925, 2008.

[18] Tamas Szekely and Kevin Burrage. Stochastic simulation in systems biology. *Computational and structural biotechnology journal*, 12(20):14–25, 2014.

[19] Marcello Buiatti and Giuseppe Longo. Randomness and multilevel interactions in biology. *Theory in Biosciences*, 132(3):139–158, 2013.

[20] Mukhtar Ullah and Olaf Wolkenhauer. Stochastic approaches in systems biology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 2(4):385–397, 2010.

[21] German Rivas and Allen P Minton. Macromolecular crowding in vitro, in vivo, and in between. *Trends in Biochemical Sciences*, 2016.

[22] Nicholas Rescher. *Many-valued logic*. Springer, 1968.

[23] Christoph Mussel, Martin Hopfensitz, and Hans A Kestler. Boolnet–an r package for generation, reconstruction and analysis of boolean networks. *Bioinformatics*, 26(10):1378–1380, 2010.

[24] Assieh Saadatpour, Istvan Albert, and Reka Albert. Attractor analysis of asynchronous boolean models of signal transduction networks. *Journal of theoretical biology*, 266(4):641–656, 2010.

[25] Lotfi A Zadeh. Fuzzy sets. *Information and control*, 8(3):338–353, 1965.

[26] Evangelia Koutsogiannouli, Athanasios G Papavassiliou, and Nikolaos A Papanikolaou. Complexity in cancer biology: is systems biology the answer? *Cancer medicine*, 2(2):164–177, 2013.

[27] Christof Koch. Modular biological complexity. *Science*, 337(6094):531–532, 2012.

[28] S Yu Jessica and Neda Bagheri. Multi-class and multi-scale models of complex biological phenomena. *Current opinion in biotechnology*, 39:167–173, 2016.

[29] Joseph Walpole, Jason A Papin, and Shayn M Peirce. Multiscale computational models of complex biological systems. *Annual review of biomedical engineering*, 15:137, 2013.

[30] Jasmin Fisher and Nir Piterman. The executable pathway to biological networks. *Briefings in functional genomics*, 9(1):79–92, 2010.

[31] EO Voit, Z Qi, and GW Miller. Steps of modeling complex biological systems. *Pharmacopsychiatry*, 41(S 01):S78–S84, 2008.

[32] Hans Peter Fischer. Mathematical modeling of complex biological systems: from parts lists to understanding systems behavior. *Alcohol Research & Health*, 31(1):49, 2008.

[33] Christos G Mihos, Andres M Pineda, and Orlando Santana. Cardiovascular effects of statins, beyond lipid-lowering properties. *Pharmacological research*, 88:12–19, 2014.

[34] C Mary Schooling, Shiu Lun Au Yeung, and Gabriel M Leung. Why do statins reduce cardiovascular disease more than other lipid modulating therapies? *European journal of clinical investigation*, 44(11):1135–1140, 2014.

[35] Lotfi Asker Zadeh. Fuzzy logic. 1988.

[36] Arnaud Poret, Claudio Monteiro Sousa, and Jean-Pierre Boissel. Enhancing boolean networks with fuzzy operators and edge tuning. *arXiv preprint arXiv:1407.1135*, 2014.

[37] Melody K Morris, Julio Saez-Rodriguez, David C Clarke, Peter K Sorger, and Douglas A Lauffenburger. Training signaling pathway maps to biochemical data with constrained fuzzy logic: quantitative analysis of liver cell responses to inflammatory stimuli. *PLoS Comput Biol*, 7(3):e1001099, 2011.

[38] Bree B Aldridge, Julio Saez-Rodriguez, Jeremy L Muhlich, Peter K Sorger, and Douglas A Lauffenburger. Fuzzy logic analysis of kinase pathway crosstalk in tnf/egf/insulin-induced signaling. *PLoS Comput Biol*, 5(4):e1000340, 2009.

[39] Xiaowei Zhu, Mark Gerstein, and Michael Snyder. Getting connected: analysis and principles of biological networks. *Genes & Development*, 21(9):1010–1024, 2007.

[40] Frank Emmert-Streib, Matthias Dehmer, and Benjamin Haibe-Kains. Gene regulatory networks and their applications: understanding biological and medical problems in terms of networks. *Frontiers in cell and developmental biology*, 2:38, 2014.