BoolNetPerturb

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Introduction

This tutorial explains how to use BoolNetPerturb in conjuction with BoolNet to study Boolean regulatory networks and the biological implications of this analysis.

This tutorial supposes that the reader is familiar with the basic concepts of:

- **Boolean regulatory networks**. There are a lot of basic introductions to the topic like: Kaplan & Glass, 1995, Azpeitia 2011 and Albert 2014. CoLoMoTo also published a more advanced review CoLoMoTo 2015.
- **R programming language**. A good starting point is the R programming course at Coursera.
- **Molecular biology**. Contact your local biologist.
- **Robustness**. The book Wagner 2005 was a great inspiration for this work.

The standar format for logical regulatory networks is SBML-qual.

Installation

The library can be installed from github using `devtools`

```
library(devtools)
install_github("mar-esther23/boolnet-perturb", force = TRUE)
```

Regulatory Networks

Regulatory Networks (RN) are a useful tool for studying the cellular behaivor and robustness of biological systems in response to different kinds of perturbations[Colomoto 2015]. RN integrate the available information of the molecular regulation to predict cellular level phenomena using a mathematical formalism. RN are deterministic dynamic systems. RN consist of nodes -that represent genes, proteins, or other biological processes- and edges -that represent the regulatory interactions among the nodes. Using this information, it is possible to construct functions that describe the state of the nodes depending on the state of its regulators. The value of the node represents wether the gene or protein is active or inactive in the biological system. The effect of the environment can be included in this models as input nodes.

The functions of the network are evaluated to obtain the attractors of the network. Attractors represent stable states in the dynamics of the network and have been related to cell types or biological processes like the cell cycle[Kaufman 1969, Azpeitia 2011, Albert 2014] [Figure 1]. RN let us simulate multiple types of perturbations depending in which part of the RN we alter. We can say that an *attractor* is stable to a perturbation if the RN returns to the same attractor, or plastic if it transitions to a different attractor. The robustness of the *system* is the result of the stability and plasticity of all its attractors. The robustness of the system can differ depending on the perturbation. In this way, robustness is a characteristic of the system that emerges from the interactions between the components of the system.

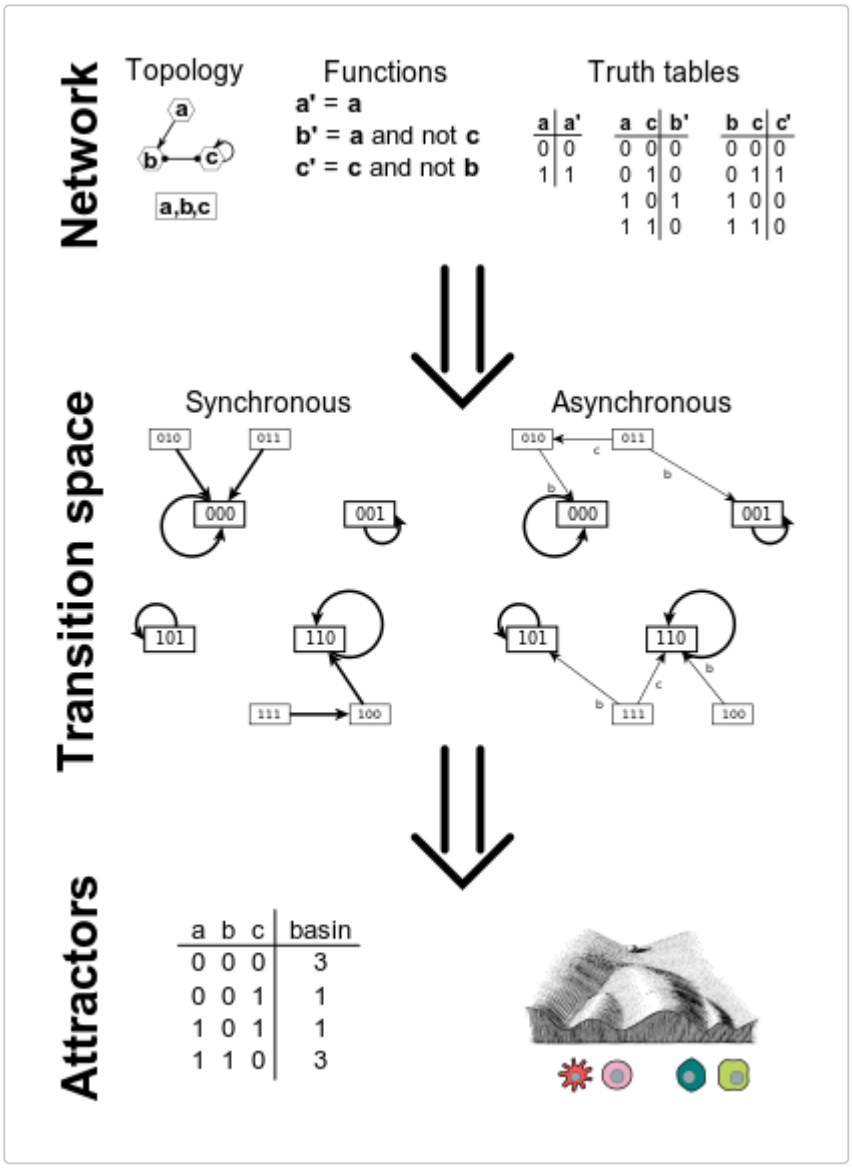
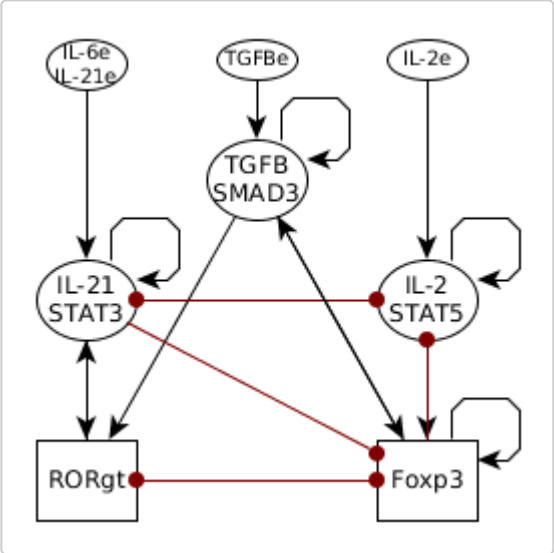


Figure 1: Boolean regulatory networks. A regulatory network consist of nodes, its interactions, and the Boolean functions that regulate the value of each node. The state of each node can be updated using this functions, to obtain the transition space. The attractors of the network correspond to biological cell types.

Biological system: Th17/Treg network

In this work we will use the Th17/Treg regulatory network as an example. This network is a subset of the CD4+ T cell regulatory network that has alredy been published and analysed using this methodology [Martinez-Sanchez 2015]. CD4 + T cells are fundamental for the adaptive immune response. They integrate the signals of the environment and differentiate from naive (Th0) cells into different cell types (Th17, iTreg, Th3, etc), which activate different parts of the immune system. In particular, Th17 cells have been associated with the inflammatory response and iTreg cells with the regulation of the inflammatory response.

CD4+ T cells begin as naïve Th0 cells, which do not express a transcription factor. These cells are activated by antigen presentation and differentiate depending in the cytokines in the environment. In the presence of IL-6 or IL-21 and TGFβ Th0 cells differentiate into Th17 cells and express RORγt, IL-21 and IL-17. In the presence of IL-2 and TGFβ Th0 cells differentiate into iTreg cells and express Foxp3 and TGFβ. There also exist Th3 cells, which are TGFβ+Foxp3-. These cytokines and transcription factors regulate each other and their relationships can be visualized a as graph[Zhu 2010, Carrier 2007].



The Th17/iTreg network can be expressed as a set of boolean functions obtained from the known interactions among the cytokines and transcription factors. Cytokines are intrinsic if produced by the CD4+ T cell, and extrinsic if produced by other cells of the immune system. We will distinguish extrinsic cytokines by adding *e* at the end of the cytokine name.

```
library("BoolNetPerturb")

data(netTh17Treg)
netTh17Treg
#> Boolean network with 8 genes
#>
#> Involved genes:
#> IL2 RORGT STAT3 FOXP3 TGFB IL2e IL21e TGFBe
#>
#> Transition functions:
#> IL2 = (IL2e | (IL2 &  ! FOXP3)) &  ! STAT3
#> RORGT = (STAT3 & TGFB) &  ! FOXP3
#> STAT3 = (IL21e | STAT3 | RORGT) &  ! IL2
#> FOXP3 = (IL2 & (TGFB | FOXP3)) &  ! (STAT3 | RORGT)
#> TGFB = TGFBe | ((TGFB | FOXP3) &  ! STAT3 )
#> IL2e = IL2e
#> IL21e = IL21e
#> TGFBe = TGFBe
```

Labels and cell types

As the state of the network is updated using the functions, the network will reach a previously visited state called an attractor. Attractors can be steady states or cycles. The set of states that lead to an attractor is called the basin of the attractor. Attractors represent cell types or biological processes. The function `attractorToDataframe()` allows us to see the attractor as a dataframe

```
attr <- getAttractors(netTh17Treg)
                                    (attr, Boolean = TRUE)
head(attr.df)
```

```
#>   attractor state IL2 RORGT STAT3 FOXP3 TGFB IL2e IL21e TGFBe
#> 1         1     1   0     0     0     0    0    0     0     0
#> 2         2     1   1     0     0     0    0    0     0     0
#> 3         3     1   0     0     1     0    0    0     0     0
#> 4         4     1   0     0     0     0    1    0     0     0
#> 5         5     1   1     0     0     0    0    1     0     0
#> 6         6     1   0     0     1     0    0    1     0     0
```

It is very important to verify that all the expected cell types appear in our attractors, if they are not present we might be missing interactions. It is also important to see if there are attractors that do not correspond to known cell types, as they may be predictions or show errors in the construction of the network. However, this can be complicated if the network is very large or has a large number of input nodes, as we may get multiple atractors that are biologicaly equivalent. A possible solution is to use known biological markers. If a cell type is charaterized by teh presence of absence of certain molecules we can use that information to create a Boolean function that will allow us to label the attractors.

For example, in the case of the Th17/Treg network, we will consider that an attractor corresponds to a cell type if both the master transcription factor and characteristic cytokine are active.

```
data("labelsTh17Treg")
labelsTh17Treg
#>   labels                    rules
#> 1    Th0  !(RORGT | FOXP3 | TGFB)
#> 2   Th17            RORGT & STAT3
#> 3   Treg             FOXP3 & TGFB
#> 4    Th3 TGFB & ! (RORGT | FOXP3)
#> 5 RORGT+          RORGT & ! STAT3
#> 6 FOXP3+           FOXP3 & ! TGFB
```

Using this rules we can label the attractors of the network using `labelAttractors()`. By default the labels of each state of a cyclic attractor are joined with "/". The function `labelState()` does the same for a single state.

```
labels <- labelAttractors(attr, labelsTh17Treg)
table(labels)
#> labels
#> RORGT+/Th3        Th0    Th0/Th0       Th17        Th3       Treg
#>          2          9          1          4          2          4
```

Robustness and plasticity in biological systems

Organisms develop in a changing world where they are exposed to intrinsic and extrinsic perturbations. Because of this perturbations, they need to be both resilient and adaptable, depending of the situation. This two behaviors coexist in all organisms, which suggests that there are common mechanism that underlie both robustness and plasticity.

Robustness is the capacity of an organism of maintaining its biological function in response to perturbations. A system is stable if it returns to the initial state after a perturbation, and plastic if it transitions to a new state. However, for studying robustness it is necessary to determine *what* function of the system is robust to *which* kind of perturbations[Wagner 2005].

Here we provide some tools for some of the most common perturbations that can be done to a Boolean network.

Target	Perturbation	Biological equivalent
Function	Fixed functions	Knock-out and over expressions, permanent changes in environment
Function	Transition table	Misconstruction of the network, small changes in regulation, evolvability
Dynamic	Updating	Time and hierarchy of biological processes.
Dynamic	State transitions	Transient biological behaivor.
State	Directed transient	Temporal changes in expression, transient environmental signals.
State	Stochastic	Biological stochastic processes.

Functions

Dynamic

State

Cell-fate map

Connect to other packages

BoolNet

Other

It is possible to plot the network topology dataframe with the R package `igraph`. This dataframe can also be used to import and export the network topology to other resources like the python library `networkx` or to the software `Cytoscape`.

It is possible to export the network functions as an SBML file using the `BoolNet` function `toSBML()`.

References