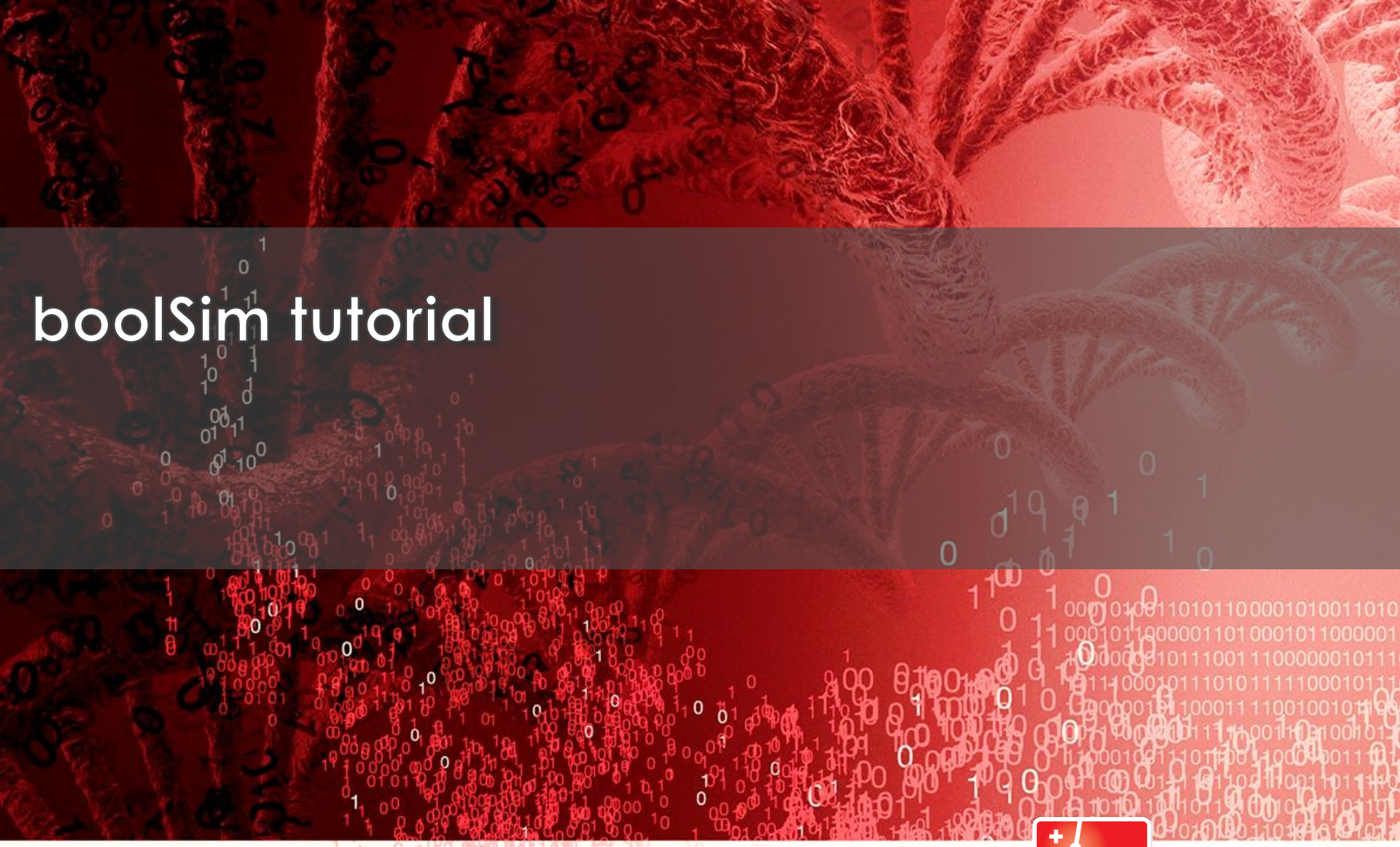
boolSim tutorial



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Introduction

During this tutorial, we will use the T-helper model proposed by A. Naldi and coauthors in 2010[1]. The full version of the model can be found in boolSim format:

`Desktop/Tutorial/boolSim/Th_Naldi_etal_2010.net`

and the reduced version:

`Desktop/Tutorial/boolSim/Th_Naldi_etal_2010_reduced.net`

The reduced model has two multivalued nodes (IL2R and STAT5). Since boolSim can only be used with Boolean models, each multivalued node had to be replaced by two Boolean nodes (IL2R_b1, IL2R_b2, STAT5_b1 and STAT5_b2), with the following mapping between multivalued states and Boolean states[2]:

IL2R	↔	IL2R_b1	IL2R_b2
0	↔	0	0
1	↔	1	0
2	↔	1	1

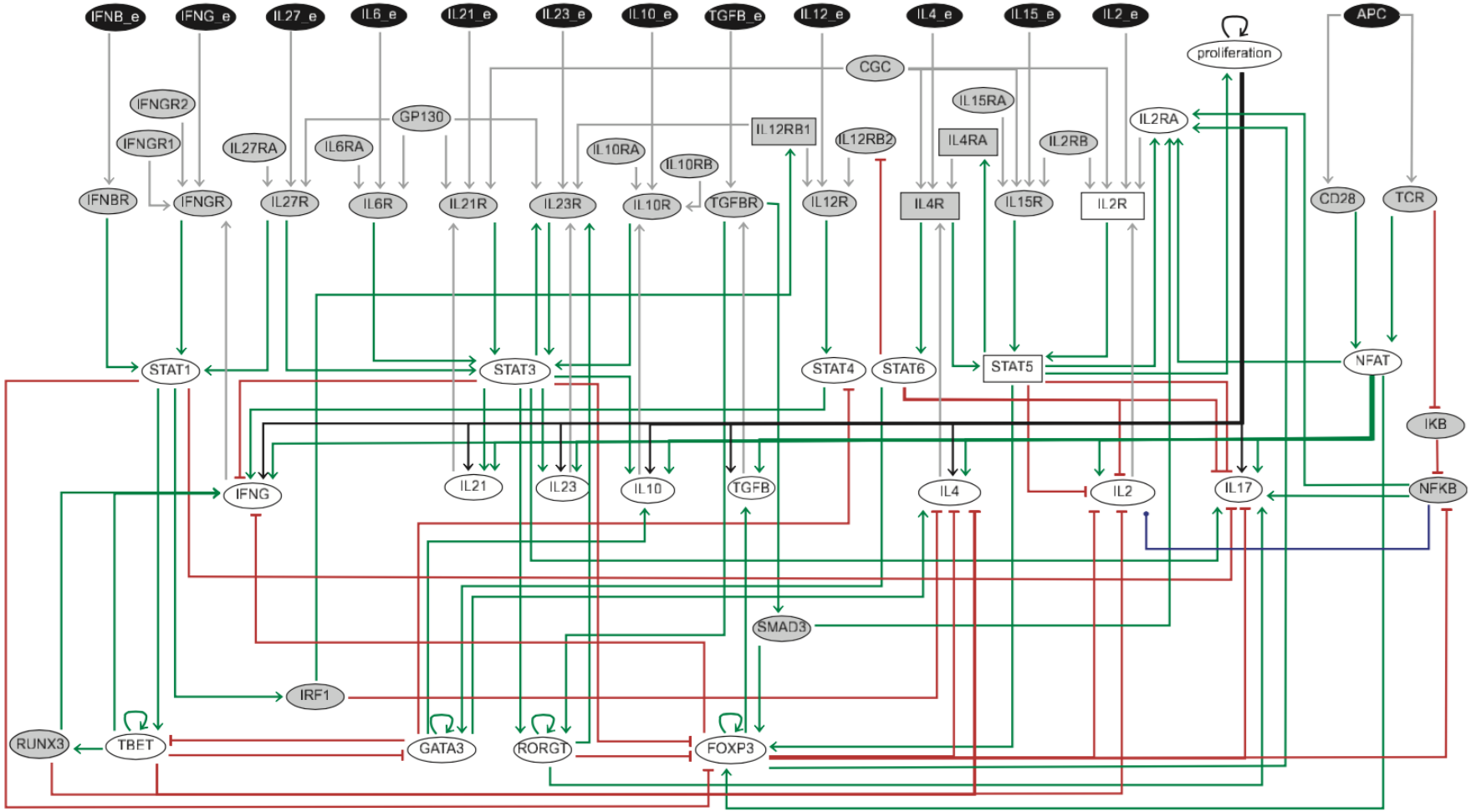
STAT5	↔	STAT5_b1	STAT5_b2
0	↔	0	0
1	↔	1	0
2	↔	1	1

These models can be downloaded from http://www.ginsim.org/node/79. To convert to boolSim format:

- Open the model with GINsim.
- Select each input node (black circular nodes) and uncheck the "input" checkbox in the modelling attributes tab.
- Export to boolSim: Menu File → Export → Boolsim.

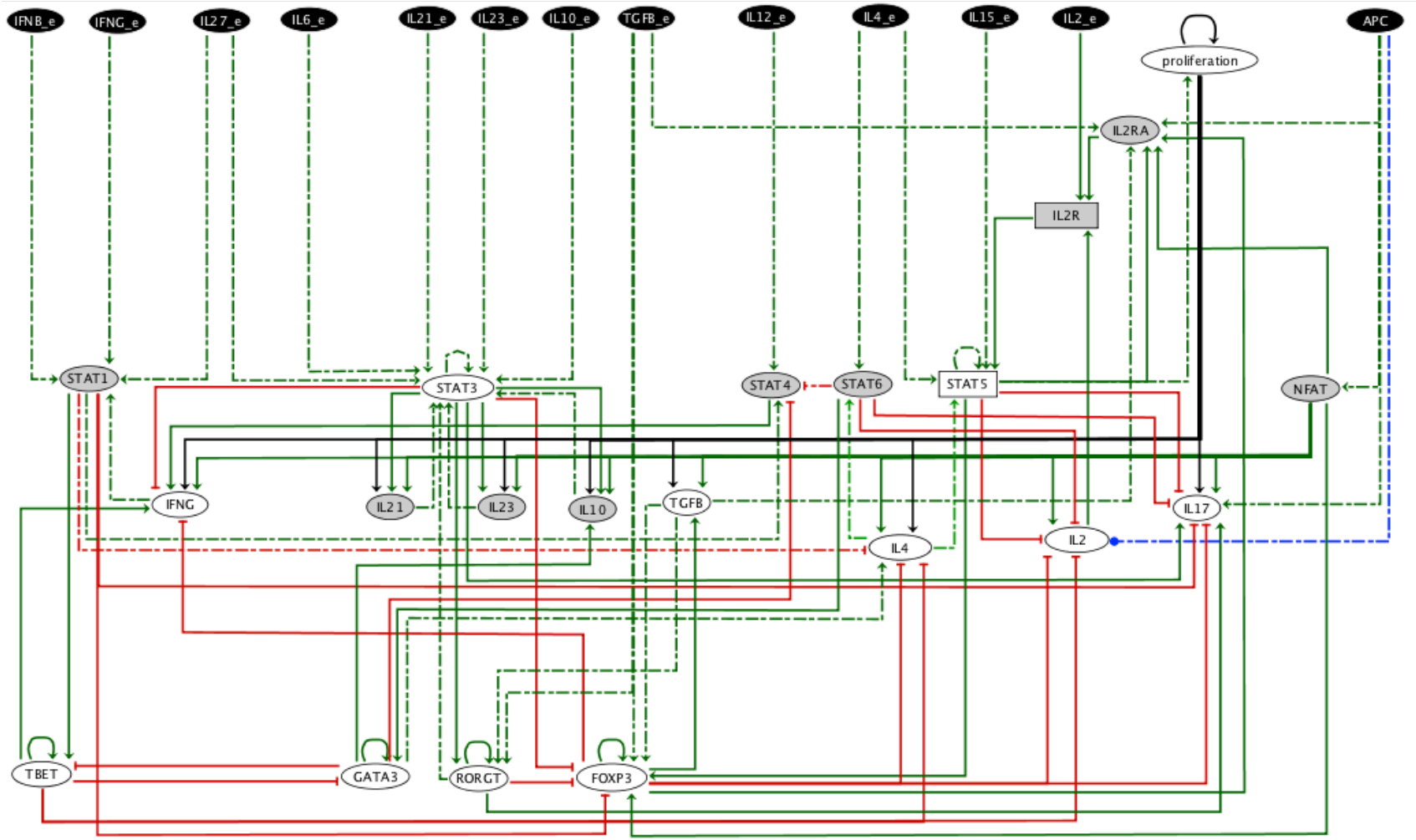
[1] Naldi et al., PLoS comp biol (2010)
[2] Didier et al., Journal of theoretical biology (2011).

T-helper model[1]



[1]Naldi et al., PLoS comp biol (2010)

Reduced T-helper[1]



[1]Naldi et al., PLoS comp biol (2010)

Preparation

boolSim is a command line program. To use it, start a terminal by double-clicking on the Terminal Emulator icon.
In the terminal, set the current working directory to `Desktop/Tutorial/boolSim/`:

```
$ cd Desktop/Tutorial/boolSim/
```

This directory contains all files which will be used during this tutorial.

To use boolSim with perturbation experiments, a directory `results/` must exist in the current directory. To create it:

```
$ mkdir results/
```

Attractors

Problem 1:

1. Use boolSim to find all asynchronous attractors of the T-helper model in the absence of external stimulation.

2. What are the stable cell lineages predicted by the model in the absence of external stimulation?
   Use the following table to associate each attractor to a cell lineage:

Cell lineage	Master regulators			
	TBET	GATA3	RORGT	FOXP3
Th0	0	0	0	0
Th1	**1**	0	0	0
Th17	0	0	**1**	0
Th2	0	**1**	0	0
Treg	0	0	0	**1**
Th1 Foxp3+	**1**	0	0	**1**
Th2 Foxp3+	0	**1**	0	**1**
Treg RORγt+	0	0	**1**	**1**
Th1 Foxp3+ RORγt+	**1**	0	**1**	**1**
Th2 Foxp3+ RORγt+	0	**1**	**1**	**1**
Th1 RORγt+	**1**	0	**1**	0
Th2 RORγt+	0	**1**	**1**	0

Naldi et al., PLoS comp biol (2010)

Solution:

1. In the T-helper network, nodes corresponding to external stimulations are source nodes (not regulated). In boolSim, these nodes are by default in state 0. Therefore we simply have to find the attractors of the unperturbed T-helper network. To get asynchronous attractors, boolSim must be run with argument –p 3

```
$ boolSim -f Th_Naldi_etal_2010_reduced.net -p 3 -o results/attractor
```

```
parsing network file
geneDbOrg=36 geneDbReduced=3
Attractor 1 ::: number of states = 1
Attractor 2 ::: number of states = 1
Attractor 3 ::: number of states = 1
Attractor 4 ::: number of states = 1
Attractor 5 ::: number of states = 1
Attractor 6 ::: number of states = 1
States of the attractor 1 are written in the file results/attractor_1
States of the attractor 2 are written in the file results/attractor_2
States of the attractor 3 are written in the file results/attractor_3
States of the attractor 4 are written in the file results/attractor_4
States of the attractor 5 are written in the file results/attractor_5
States of the attractor 6 are written in the file results/attractor_6
```

2. Attractors are saved in files attractors_1,2,...,6

```
$ cat results/attractor_1
```

```
Gene Name/State No.    S_1
IL2R_b1                  0
IL2_e                    0
IL2R_b2                  0
IL2                      0
IL2RA                    0
APC                      0
TGFB_e                   0
TGFB                     0
FOXP3                    0
NFAT                     0
STAT5_b1                 0
IFNG                     0
TBET                     1
STAT3                    0
STAT4                    0
proliferation            0
STAT6                    0
IL4                      0
GATA3                    0
STAT1                    0
IL10                     0
IL21                     0
IL23                     0
RORGT                    0
IFNB_e                   0
IFNG_e                   0
IL27_e                   0
IL6_e                    0
IL10_e                   0
```

Th1

```
$ cat results/attractor_2
```

```
Gene Name/State No.    S_1
IL2R_b1                  0
IL2_e                    0
IL2R_b2                  0
IL2                      0
IL2RA                    0
APC                      0
TGFB_e                   0
TGFB                     0
FOXP3                    0
NFAT                     0
STAT5_b1                 0
IFNG                     0
TBET                     1
STAT3                    0
STAT4                    0
proliferation            1
STAT6                    0
IL4                      0
GATA3                    0
STAT1                    0
IL10                     0
IL21                     0
IL23                     0
RORGT                    0
IFNB_e                   0
IFNG_e                   0
IL27_e                   0
IL6_e                    0
IL10_e                   0
```

Th1

```
$ cat results/attractor_3
```

```
Gene Name/State No.    S_1
IL2R_b1                  0
IL2_e                    0
IL2R_b2                  0
IL2                      0
IL2RA                    0
APC                      0
TGFB_e                   0
TGFB                     0
FOXP3                    0
NFAT                     0
STAT5_b1                 0
IFNG                     0
TBET                     0
STAT3                    0
STAT4                    0
proliferation            0
STAT6                    0
IL4                      0
GATA3                    0
STAT1                    0
IL10                     0
IL21                     0
IL23                     0
RORGT                    0
IFNB_e                   0
IFNG_e                   0
IL27_e                   0
IL6_e                    0
IL10_e                   0
```

Th0

$ cat results/attractor_4

Gene Name/State No.	S_1
IL2R_b1	0
IL2_e	0
IL2R_b2	0
IL2	0
IL2RA	0
APC	0
TGFB_e	0
TGFB	0
FOXP3	0
NFAT	0
STAT5_b1	0
IFNG	0
TBET	0
STAT3	0
STAT4	0
proliferation	1
STAT6	0
IL4	0
GATA3	0
STAT1	0
IL10	0
IL21	0
IL23	0
RORGT	0
IFNB_e	0
IFNG_e	0
IL27_e	0
IL6_e	0
IL10_e	0

Th0

$ cat results/attractor_5

Gene Name/State No.	S_1
IL2R_b1	0
IL2_e	0
IL2R_b2	0
IL2	0
IL2RA	0
APC	0
TGFB_e	0
TGFB	0
FOXP3	0
NFAT	0
STAT5_b1	0
IFNG	0
TBET	0
STAT3	0
STAT4	0
proliferation	0
STAT6	0
IL4	0
GATA3	1
STAT1	0
IL10	0
IL21	0
IL23	0
RORGT	0
IFNB_e	0
IFNG_e	0
IL27_e	0
IL6_e	0
IL10_e	0

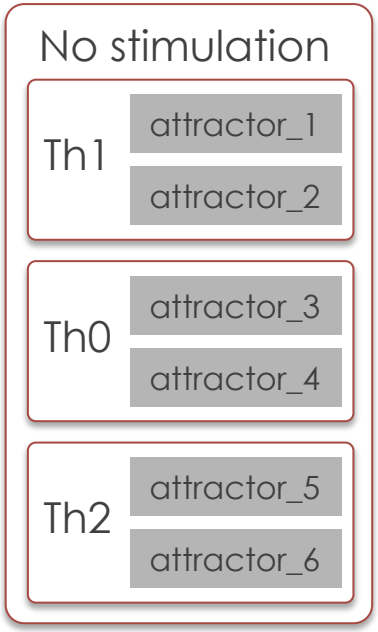
Th2

$ cat results/attractor_6

Gene Name/State No.	S_1
IL2R_b1	0
IL2_e	0
IL2R_b2	0
IL2	0
IL2RA	0
APC	0
TGFB_e	0
TGFB	0
FOXP3	0
NFAT	0
STAT5_b1	0
IFNG	0
TBET	0
STAT3	0
STAT4	0
proliferation	1
STAT6	0
IL4	0
GATA3	1
STAT1	0
IL10	0
IL21	0
IL23	0
RORGT	0
IFNB_e	0
IFNG_e	0
IL27_e	0
IL6_e	0
IL10_e	0

Th2

To summarize, without external stimulation, the T-helper model predicts three stable cell lineages, each represented by two attractors (differing only in proliferation = 0 or 1)



Perturbation experiments

Problem 2:

Th0 should differentiate into Th1 when stimulated with APC+IL2+IFNG. Check that the model reproduces this behavior.

1. Create an experiment file with two stages:
   - Stage 1: no stimulation
   - Stage 2: APC=1, IL2_e=1, IFNG_e=1 (pro Th1 conditions)

2. Associate each attractor to a cell lineage and draw the attractors transition graph .

Cell lineage	Master regulators			
	TBET	GATA3	RORGT	FOXP3
Th0	0	0	0	0
Th1	**1**	0	0	0
Th17	0	0	**1**	0
Th2	0	**1**	0	0
Treg	0	0	0	**1**
Th1 Foxp3+	**1**	0	0	**1**
Th2 Foxp3+	0	**1**	0	**1**
Treg RORγt+	0	0	**1**	**1**
Th1 Foxp3+ RORγt+	**1**	0	**1**	**1**
Th2 Foxp3+ RORγt+	0	**1**	**1**	**1**
Th1 RORγt+	**1**	0	**1**	0
Th2 RORγt+	0	**1**	**1**	0

Naldi et al., PLoS comp biol (2010)

Solution:

1. Create the experiment file `nostimulation_proTh1.exp`

```
$ cat nostimulation_proTh1.exp
```

```
2          ← 2 stages
0 0 0      ← Stage 1: 0 node fixed to 0, 0 node fixed to 1, 0 unconstrained node
0 3 0      ← Stage 2:  0 node fixed to 0, 3 nodes fixed to 1, 0 unconstrained node
APC        ← APC is fixed to state 1
IL2_e      ← IL2_e is fixed to state 1
IFNG_e     ← IFNG_e is fixed to state 1
```

2. Create the directory `results/`

```
$ mkdir results/
```

Run boolSim:

```
$ boolSim -f Th_Naldi_etal_2010_reduced.net -e nostimulation_proTh1.exp
```

```
parsing network file
geneDbOrg=36 geneDbReduced=25
processing experiment file level 1
-- reachability --
SS_1_1  ---->  SS_2_7
SS_1_2  ---->  SS_2_7
SS_1_3  ---->  SS_2_7
SS_1_4  ---->  SS_2_7
SS_1_5  ---->  SS_2_5
SS_1_6  ---->  SS_2_5
Results are written in the file 'results/reach_.txt'
```

Open the output file `results/reach_.txt`

```
$ cat results/reach_.txt
```

```
##############################################

###### unperturbed network ##################

States of the attractor 1 are written in the file results/_SS_1_1.txt
States of the attractor 2 are written in the file results/_SS_1_2.txt
States of the attractor 3 are written in the file results/_SS_1_3.txt
States of the attractor 4 are written in the file results/_SS_1_4.txt
States of the attractor 5 are written in the file results/_SS_1_5.txt
States of the attractor 6 are written in the file results/_SS_1_6.txt
##############################################

#######  APC,IFNG_e,IL2_e, over-expressed  #######

States of the attractor 1 are written in the file results/_SS_2_1.txt
States of the attractor 2 are written in the file results/_SS_2_2.txt
States of the attractor 3 are written in the file results/_SS_2_3.txt
States of the attractor 4 are written in the file results/_SS_2_4.txt
States of the attractor 5 are written in the file results/_SS_2_5.txt
States of the attractor 6 are written in the file results/_SS_2_6.txt
States of the attractor 7 are written in the file results/_SS_2_7.txt
States of the attractor 8 are written in the file results/_SS_2_8.txt
States of the attractor 9 are written in the file results/_SS_2_9.txt
***** Reachability Analysis *****
SS_1_1  ---->  SS_2_7
SS_1_2  ---->  SS_2_7
SS_1_3  ---->  SS_2_7
SS_1_4  ---->  SS_2_7
SS_1_5  ---->  SS_2_5
SS_1_6  ---->  SS_2_5
```

Stage 1 attractors are saved in
`results/_SS_1_*.txt`.
Check that attractors 2 and 4 correspond to Th0 cells (TBET=0, GATA3=0, RORGT=0, FOXP3=0):

```
$ grep -e TBET -e GATA3 -e RORGT -e FOXP3 results/_SS_1_2.txt
```

```
FOXP3                   0
TBET                    0
GATA3                   0
RORGT                   0
```

```
$ grep -e TBET -e GATA3 -e RORGT -e FOXP3 results/_SS_1_4.txt
```

```
FOXP3                   0
TBET                    0
GATA3                   0
RORGT                   0
```

Similarly, attractors 1 and 3 correspond to Th1 (TBET=1) and attractors 5 and 6 to Th2 (GATA3=1)

Note that the order of attractors is arbitrary: attractors 3 and 4 obtained in problem 1 correspond respectively to attractors 2 and 4 in problem 2.

Stage 2 attractors are saved in `results/_SS_2_*.txt`.
Based on the states of nodes TBET, GATA3, RORGT and FOXP3 check that attractor 1 corresponds to Th1

```
$ grep -e TBET -e GATA3 -e RORGT -e FOXP3 results/_SS_2_1.txt
```

```
FOXP3                   0
TBET                    1
GATA3                   0
RORGT                   0
```

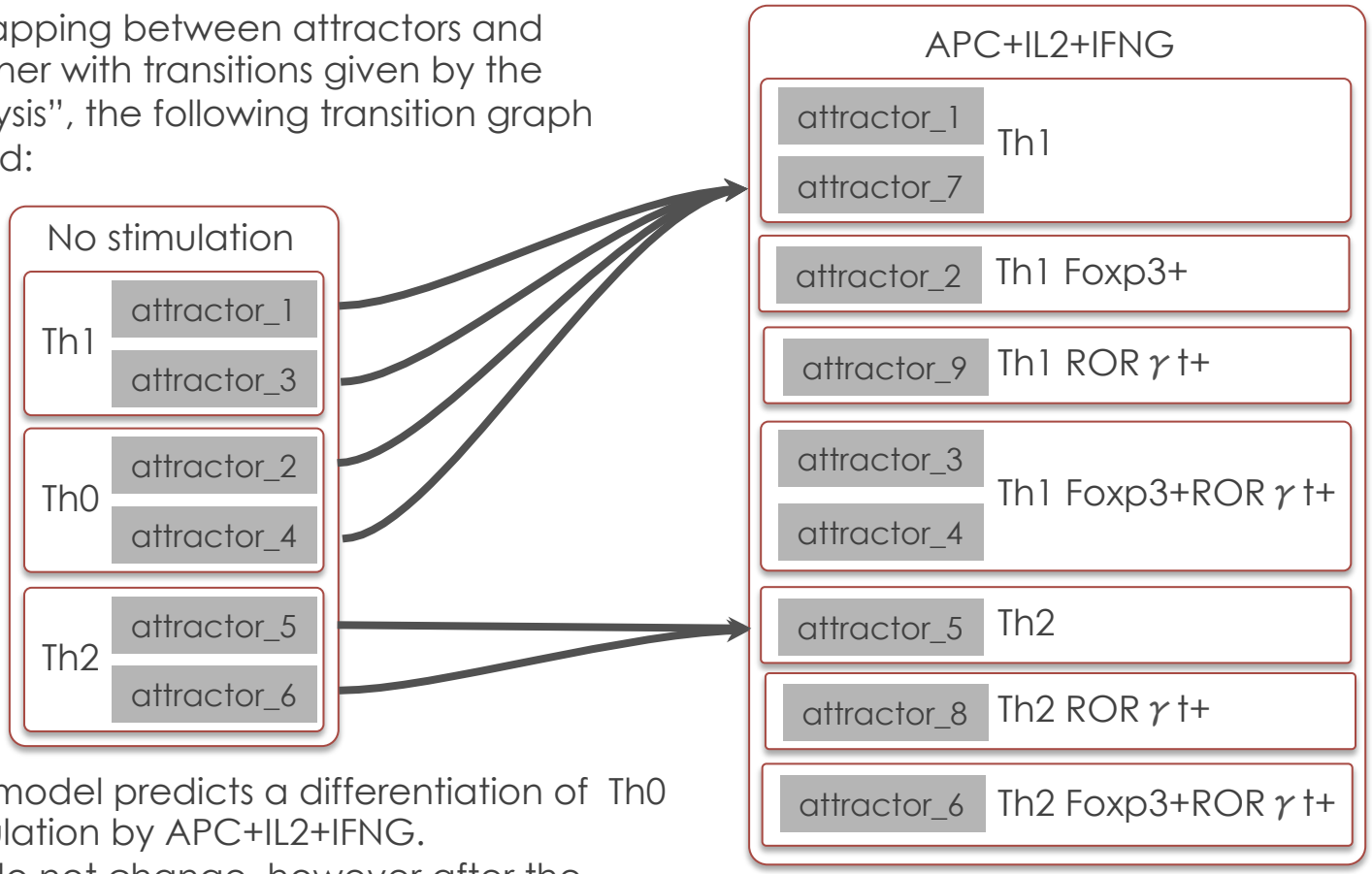
Repeating the same procedure for all attractors should give the following mapping between attractors and cell types:

Attractor		Cell type	Signature
1	↔	Th1	TBET=1, GATA3=0, RORGT=0, FOXP3=0
2	↔	Th1 Foxp3+	TBET=1, GATA3=0, RORGT=0, FOXP3=1
3	↔	Th1 Foxp3+ ROR γ t+	TBET=1, GATA3=0, RORGT=1, FOXP3=1
4	↔	Th1 Foxp3+ ROR γ t+	TBET=1, GATA3=0, RORGT=1, FOXP3=1
5	↔	Th2	TBET=0, GATA3=1, RORGT=0, FOXP3=0
6	↔	Th2 Foxp3+ ROR γ t+	TBET=0, GATA3=1, RORGT=1, FOXP3=1
7	↔	Th1	TBET=1, GATA3=0, RORGT=0, FOXP3=0
8	↔	Th2 ROR γ t+	TBET=0, GATA3=1, RORGT=1, FOXP3=0
9	↔	Th1 ROR γ t+	TBET=1, GATA3=0, RORGT=1, FOXP3=0

To build the transition graph, go back to the file
`results/reach_.txt`
The part “reachability analysis” lists the transitions from attractors in stage 1 to attractors in stage 2:

```
States of the attractor 2 are written in the file results/_SS_2_2.txt
States of the attractor 3 are written in the file results/_SS_2_3.txt
States of the attractor 4 are written in the file results/_SS_2_4.txt
States of the attractor 5 are written in the file results/_SS_2_5.txt
States of the attractor 6 are written in the file results/_SS_2_6.txt
States of the attractor 7 are written in the file results/_SS_2_7.txt
States of the attractor 8 are written in the file results/_SS_2_8.txt
States of the attractor 9 are written in the file results/_SS_2_9.txt
***** Reachability Analysis *****
SS_1_1  ---->  SS_2_7
SS_1_2  ---->  SS_2_7
SS_1_3  ---->  SS_2_7
SS_1_4  ---->  SS_2_7
SS_1_5  ---->  SS_2_5
SS_1_6  ---->  SS_2_5
```

Combining the mapping between attractors and phenotypes together with transitions given by the "reachability analysis", the following transition graph should be obtained:



As expected, the model predicts a differentiation of Th0 into Th1 after stimulation by APC+IL2+IFNG.
Th1 and Th2 cells do not change, however after the stimulation Th1 becomes activated (i.e. expressing NFAT and producing lineage-specific cytokines) and Th2 becomes anergic (i.e. expressing NFAT but no lineage-specific cytokine).
Notice the apparition of other stable cell types, which cannot be reached directly from the stable cell types obtained without stimulation.

Perturbation experiments

Problem 3:

Check that the model predicts a differentiation of Th0 into Th17 upon stimulation with APC +IL6+TGFB. Is the resulting Th17 cell type stable when placed in proTh1 conditions (APC+IL2+IFNG)?

1. Create an experiment file with three stages:
   - Stage 1: no stimulation
   - Stage 2: APC=1, IL6_e=1, TGFB_e=1 (pro Th17 conditions)
   - Stage 3: APC=1, IL2_e=1, IFNG_e=1 (pro Th1 conditions)

2. Associate each attractor to a cell lineage and draw the attractors transition graph.

Cell lineage	Master regulators			
	TBET	GATA3	RORGT	FOXP3
Th0	0	0	0	0
Th1	**1**	0	0	0
Th17	0	0	**1**	0
Th2	0	**1**	0	0
Treg	0	0	0	**1**
Th1 Foxp3+	**1**	0	0	**1**
Th2 Foxp3+	0	**1**	0	**1**
Treg RORγt+	0	0	**1**	**1**
Th1 Foxp3+ RORγt+	**1**	0	**1**	**1**
Th2 Foxp3+ RORγt+	0	**1**	**1**	**1**
Th1 RORγt+	**1**	0	**1**	0
Th2 RORγt+	0	**1**	**1**	0

Naldi et al., PLoS comp biol (2010)

Solution:

1. Create the experiment file `nostimulation_proTh17_proTh1.exp`

```
$ cat nostimulation_proTh17_proTh1.exp
```

```
3          ← 3 stages
0 0 0      ← Stage 1: 0 node fixed to 0, 0 node fixed to 1, 0 unconstrained node
0 3 0      ← Stage 2:  0 node fixed to 0, 3 nodes fixed to 1, 0 unconstrained node
APC        ← APC is fixed to state 1
IL6_e      ← IL6_e is fixed to state 1
TGFB_e     ← TGFB_e is fixed to state 1
0 2 2      ← Stage 3:  0 node fixed to 0, 2 nodes fixed to 1, 2 unconstrained nodes
IL2_e      ← IL2_e is fixed to state 1
IFNG_e     ← IFNG_e is fixed to state 1
IL6_e      ← IL6_e is unconstained
TGFB_e     ← TGFB_e is unconstained
```

2. boolSim will overwrite previously obtained files in the `results/` directory. To avoid this, rename the directory to `results_proTh1`

```
$ mv results/ results_proTh1/
```

2. Create the directory `results/`

```
$ mkdir results/
```

Run boolSim:

```
$ boolSim -f Th_Naldi_etal_2010_reduced.net -e nostimulation_proTh17_proTh1.exp
```

```
parsing network file
geneDbOrg=36 geneDbReduced=27
processing experiment file level 1
-- reachability --
SS_1_1  ---->  SS_2_2
SS_1_2  ---->  SS_2_1
SS_1_3  ---->  SS_2_5
SS_1_4  ---->  SS_2_1
SS_1_5  ---->  SS_2_3
SS_1_5  ---->  SS_2_4
SS_1_6  ---->  SS_2_3
SS_1_6  ---->  SS_2_4
processing experiment file level 2
-- reachability --
SS_2_1  ---->  SS_3_4
SS_2_2  ---->  SS_3_4
SS_2_2  ---->  SS_3_5
SS_2_3  ---->  SS_3_9
SS_2_4  ---->  SS_3_9
SS_2_5  ---->  SS_3_4
Results are written in the file 'results/reach_.txt'
```

Stage 1 attractors are saved in `results/_SS_1_*.txt`.
Check that attractors 2 and 4 correspond to Th0 cells (TBET=0, GATA3=0, RORGT=0, FOXP3=0):

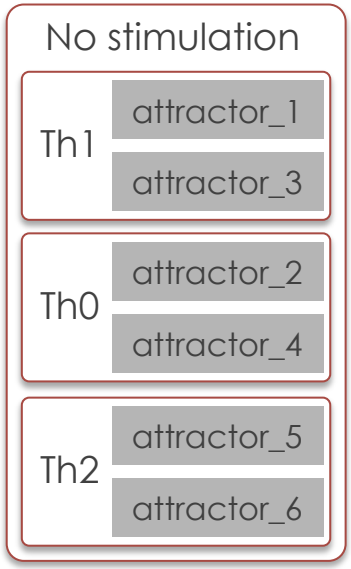
```
$ grep -e TBET -e GATA3 -e RORGT -e FOXP3 results/_SS_1_2.txt
```

```
FOXP3                   0
TBET                    0
GATA3                   0
RORGT                   0
```

```
$ grep -e TBET -e GATA3 -e RORGT -e FOXP3 results/_SS_1_4.txt
```

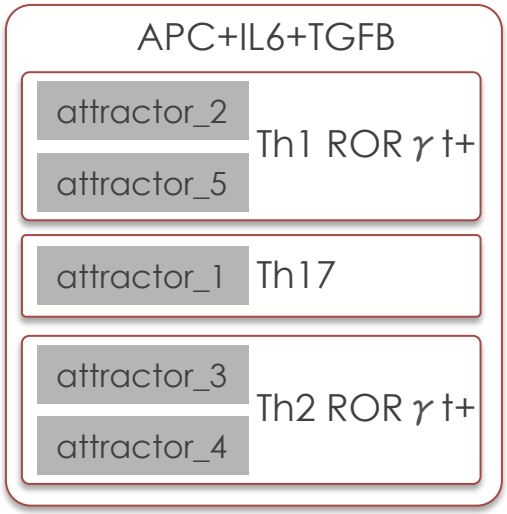
```
FOXP3                   0
TBET                    0
GATA3                   0
RORGT                   0
```

Similarly attractors 1 and 3 correspond to Th1 (TBET=1) and attractors 5 and 6 to Th2 (GATA3=1)



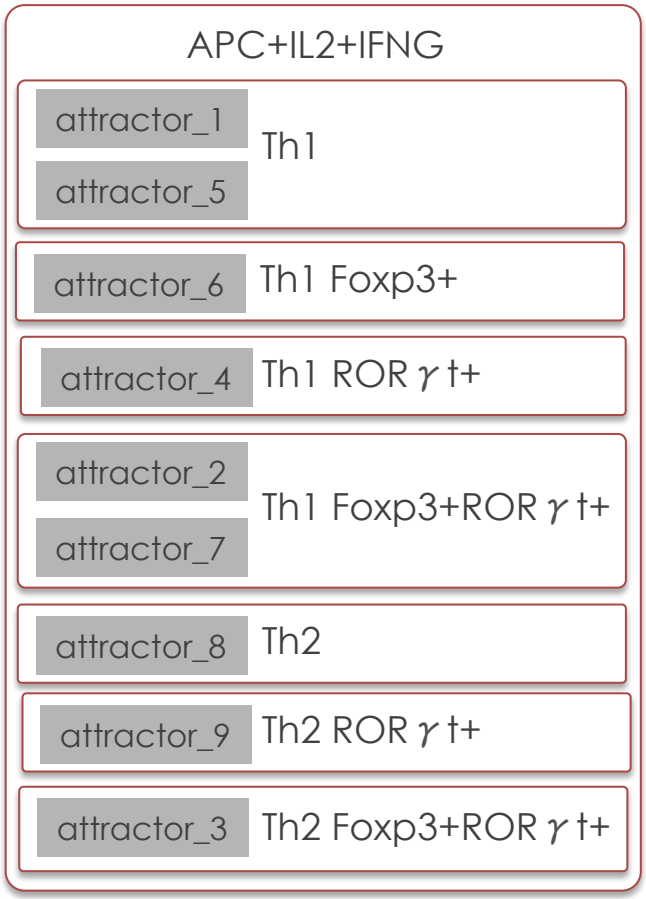
Stage 2 attractors are saved in
`results/_SS_2_*.txt`.
Based on the state of nodes TBET, GATA3, RORGT and FOXP3, the following mapping between attractors and cell type should be obtained:

Attractor		Cell type
1	↔	Th17
2	↔	Th1 ROR γ t+
3	↔	Th2 ROR γ t+
4	↔	Th2 ROR γ t+
5	↔	Th1 ROR γ t+



Stage 3 attractors are saved in
`results/_SS_3_*.txt`.
Based on the states of nodes TBET, GATA3, RORGT and FOXP3 the following mapping between attractors and cell types should be obtained:

Attractor		Cell type
1	↔	Th1
2	↔	Th1 Foxp3+ROR γ t+
3	↔	Th2 Foxp3+ROR γ t+
4	↔	Th1 ROR γ t+
5	↔	Th1
6	↔	Th1 Foxp3+
7	↔	Th1 Foxp3+ROR γ t+
8	↔	Th2
9	↔	Th2 ROR γ t+



To build the transition graph, go back to the file
`results/reach_.txt`
The first "reachability analysis" lists the transitions from attractors in stage 1 to attractors in stage 2:

```
###############################################

#######  APC,IL6_e,TGFB_e, over-expressed  #######

States of the attractor 1 are written in the file results/_SS_2_1.txt
States of the attractor 2 are written in the file results/_SS_2_2.txt
States of the attractor 3 are written in the file results/_SS_2_3.txt
States of the attractor 4 are written in the file results/_SS_2_4.txt
States of the attractor 5 are written in the file results/_SS_2_5.txt
***** Reachability Analysis *****
SS_1_1  ---->  SS_2_2
SS_1_2  ---->  SS_2_1
SS_1_3  ---->  SS_2_5
SS_1_4  ---->  SS_2_1
SS_1_5  ---->  SS_2_3
SS_1_5  ---->  SS_2_4
SS_1_6  ---->  SS_2_3
SS_1_6  ---->  SS_2_4
###############################################

#######  APC,IFNG_e,IL2_e, over-expressed  #######

States of the attractor 1 are written in the file results/_SS_3_1.txt
```

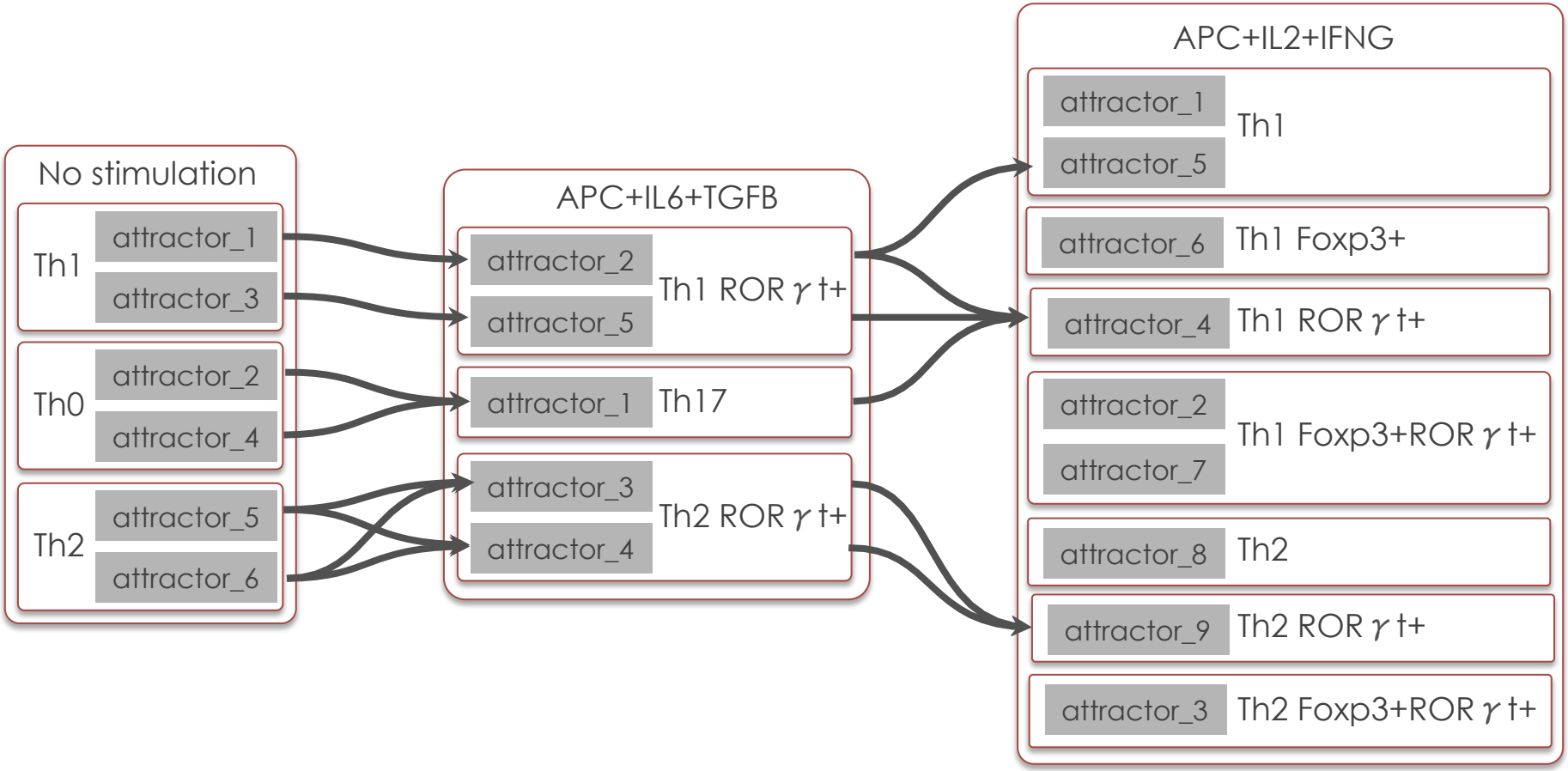
The second “reachability analysis” lists the transitions from attractors in stage 2 to attractors in stage 3:

```
SS_1_6  ---->  SS_2_3
SS_1_6  ---->  SS_2_4
###############################################

#######  APC,IFNG_e,IL2_e, over-expressed  #######

States of the attractor 1 are written in the file results/_SS_3_1.txt
States of the attractor 2 are written in the file results/_SS_3_2.txt
States of the attractor 3 are written in the file results/_SS_3_3.txt
States of the attractor 4 are written in the file results/_SS_3_4.txt
States of the attractor 5 are written in the file results/_SS_3_5.txt
States of the attractor 6 are written in the file results/_SS_3_6.txt
States of the attractor 7 are written in the file results/_SS_3_7.txt
States of the attractor 8 are written in the file results/_SS_3_8.txt
States of the attractor 9 are written in the file results/_SS_3_9.txt
***** Reachability Analysis *****
SS_2_1  ---->  SS_3_4
SS_2_2  ---->  SS_3_4
SS_2_2  ---->  SS_3_5
SS_2_3  ---->  SS_3_9
SS_2_4  ---->  SS_3_9
SS_2_5  ---->  SS_3_4
```

Combining the mapping between attractors and phenotypes together with transitions given by the "reachability analysis", the following transition graph should be obtained:



As expected, Th0 differentiates into Th17 upon APC+IL6+TGFB stimulation. When environmental conditions change to proTh1 (APC+IL2+IFNG), the model predicts that Th17 transdifferentiates into Th1 ROR γ t+.