Role and dynamics of the double toggle switch pattern in gene regulation networks

Avel GUÉNIN–CARLUT

15 février 2019

Introduction

Networks have recently been introduced in the modelling of complex systems. As they are able to capture any regularities emerging from the structured interaction of numerous elementary parts, they have been successful in catalysing research in fields as diverse as systems biology, cognitive sciences, ecology and social sciences.

The main discipline in networks-oriented complexity science is to relate dynamical or computational properties of systems to structural properties of the networks underlying them. Various exemples can be found where such an approach allowed genuine leaps in life sciences. It allowed Von Dassow et al. (2000), in the context of Wagner et al. (2007)'s concept of modularity, to help explain the structural homogeneity of morphogenic development networks in insects. It allowed Ulanowicz (2004) to quantify ecosystemic stability from trophic networks estimates, and later help apply his measure to macroeconomics (Huang and Ulanowicz, 2014).

While some macrostructural properties, characteristic of so called "scale-free networks" (Barabási and Bonabeau, 2003), seem to be universal in real life structures, mesoscopic properties seem to be more closely related to the specific function of the network and/or to the properties of its elementary bricks.

For exemple, statistically significant network motifs seem to allow a broad sorting of networks according to their function (Milo et al., 2002) : information processing (genes, neurons, and computer electronics), energy flows (food webs), and information transmission (Web). These motifs were speculated by the authors to be functionally related to the higher level network function. For exemple, feed-forward loops (which filter environmental noise) are especially common in information processing networks (be it electronic or neuronal).

However, statistical analysis is not sufficient to bridge the gap between mesoscopic motif dynamics and macroscopic network function. Functionally determinant but rare motifs would not be detected by such an approach. It is also necessary to investigate functional properties of specific network motifs to understand their integration into broader network properties.

This study investigates the dynamic properties of the double toggle switch motif in the context of genetic regulation networks. It will more specifically adress this motif's ability to retain information through time ; in other words, to implement epigenetic memory in the highly fluctuating world of biochemical dynamics.

While "memory" has a rich meaning and potentially unlimited implementations, we only focus here on its simplest and most natural traduction in the context of dynamical systems theory. It is the existence of two distinct stable states deep enough (in the potential landscape space) to be robust to fluctuations, but shallow enough to allow a switch given the relevant input.

In this regard, we will walk through the implementation of a custom mechanistic model of this motif using the classical Gillespie algorithm (which generates possible trajectories of a stochastic system). We will discuss along the way the reasons of each modelling step I took and their physical meaning (in regard of preexisting literature and expected results). Finally, we will try to characterise more precisely what "memory" means in the context of our model.

1   Model implementation

My model broadly follows what Herbach et al. (2017) developed, as their paper gave clear guidelines to implement both stochasticity and interaction (which are the two critical elements of our present analysis) into models for gene expression.

In the absence of explicit reference to another publication, every result I will use below can be attributed to their paper.

The main divergence I took in regard to their work is a significant modification of the interaction rules they presented. I added a layer of complexity by separating basal input of a given gene into separate self-activation and extrinsic stimulation terms. This decision can easily be understood by considering the intrinsic divergence of our approaches.

On one hand, their model attempts to facilitate statistical inference of the structure of in vivo, large scale gene networks. As this implies to find an analytical form for the statistical form of their model, they are considerably constrained in their account of self-activation. Besides, it is unlikely such a distinction would help them much in inferring network structure. Indeed, inferring self-activation form is so hard that they had to assume up to its direction in their model. In this context, a finer information such as its presence for specific genes cannot be inferred robustly.

On the other hand, I need to analyse the dynamical behaviour of an in silico, small-scale gene network. In this regard, having fine control over the intrinsic behaviour and extrinsic stimulation of my system is both far more relevant and far easier than in Herbach et al. (2017)'s case. I am not limited by considerations regarding the analytical properties or inferrability from real data of my model, and (as we will see below) decoupling self-activation and extrinsic stimulation helps me in designing my experimental protocol and identifying what are the key properties for memory emergence.

After this general outline of the approach I adopted, we will discuss more precisely what specific features I implemented in my model, and why they were necessary.

We will first adress how I introduced stochasticity in the analysis of gene expression in the absence of self-activation (following closely the original model), then how I accounted for the retroaction of protein concentration on gene expression (drawing inspiration but departing significantly from it). Finally we will adress the implementation of the double toggle switch itself.

1.1   Stochasticity in gene expression

I reproduced the modelling scheme used in Herbach et al. (2017), as represented figure 1.

As is standard in literature (Munsky et al., 2012), genes are modelled as having an "active" state (they produce corresponding mARN at constant rate s_0) and a "passive" state (they do not). They switch stochastically from the passive state to the active one at rate k_{on} (resp from the active to the passive state at rate k_{off}).

This way, all of the dynamics relevant to gene expression can be described as a set of chemical reactions with given propensities (the stochastic equivalent

FIGURE 1 – Scheme of the two-state model of gene expression, from Herbach et al. (2017)

of "rate constants"). This is the classical case of application of the Gillespie algorithm, which uses the distribution for next state change and next state change time (both being analytically computable from propensities, at least in the approximation where no temporal correlations exist) to generate statistically correct system trajectories.
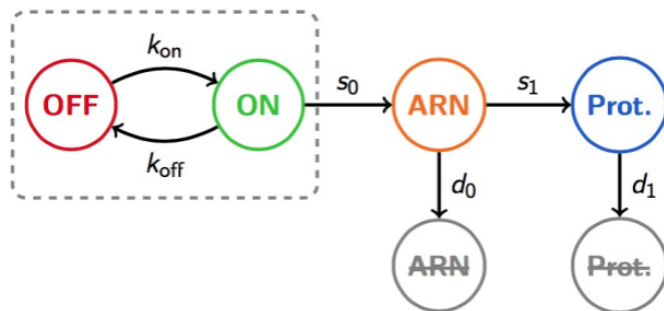
While gene activation stochasticity is crucial to the dynamics of our system, stochasticity can be ignored in other reactions. This is because both mARN and protein quantities usually evolves well above 1, which allow us to ignore the discrete aspect of their variations and approximate it to be equal to their mean rate of change. The approximation is exact in the limit where $s_0 \gg d_0$; $s_1 \gg d_1$, which is usually verified in experimental settings.

Using the continuous approximation for non-rare species, our system now switches between two deterministic differential equations depending on the value adopted by a continuous time two-state random process without memory, as appears equation 1. This makes it a Piecewise-Deterministic Markov Process, a rather understood mathematical framework. While it allows Herbach et al. (2017) to keep statistical tractability, it allows us to reduce the code complexity and focus on the relevant stochasticity sources only.

$$
\begin{aligned}
E(t) : 0 &\underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} 1 \\
M'(t) &= s_0 \cdot E(t) - d_0 \cdot M(t) \\
P'(t) &= s_1 \cdot M(t) - d_1 \cdot P(t)
\end{aligned}
\tag{1}
$$

Using a mix of Gillespie algorithm and analytical resolution of the above ordinary differential equations, I wrote a minimal library generating statistically correct trajectories for our sytem. It is articulated around the code snippet below, which generates system state and time at next gene state switch from current system state, current time and extrinsic perturbation Δ [1].

```
## Stochastic step forward
w = self.computeSwitchRates(Delta)
W = sum(w)
dt = np.random.exponential(1/W)

self.t_simu += dt

## Deterministic integration
for i in range(self.size) :
    self.m[i] /= np.exp(self.d[0]*dt)
    self.m[i] += self.s[0]*self.g[i]*(1-np.exp(-self.d[0]*
        dt))/self.d[0]
    self.p[i] /= np.exp(self.d[1]*dt)
    self.p[i] += self.s[1]*self.m[i]*(1-np.exp(self.d[1]*dt
        ))/self.d[1]

## Stochastic state change
w = [i/W for i in w]
e = np.random.choice(self.size,p=w)
self.g[e] = 1 - self.g[e]
```

FIGURE 2 – Code for generating one Gillespie time step

This simulation is validated by its dynamical behaviour, which is similar to the original. For exemple, mRNA trajectories are plotted for both our simulation and Herbach et al. (2017)'s figure 3. We can visually check that typical values, variation timescales, and bursty patterns all are conserved. Therefore, our library approximates correctly the expression dynamics of a single gene without retroaction, at least in a similar extent to the hybrid numerical scheme Herbach et al. (2017) initially implemented.

1.2 Protein retroaction

In order to study the double toggle switch motif, we must define a model for gene interaction. As modulation in genetic expression has been shown to be related to peak frequency (k_{on}) rather than peak intensity (s_0/k_{off}) (Viñuelas et al., 2013), we will account for modulation through k_{on} only.

As in Herbach et al. (2017), our mechanistic assumption is that gene expression is modulated by ligant binding dynamics, justifying the use of the classic Hill function. Because the proper form for traducing the dynamics of several interacting species is not clear (Mizeranschi et al., 2015), we have a certain level of leeway in assembling our final function.

---

1. Note that in the expression of protein levels, mRNA level is approximated to be constant. This is factually false. However, as outlined in the original paper, no short-term correlation exist between mRNA levels and system state due to de facto timescale separation between mRNA and protein dynamics. In this regard, the approximation should not (and empirically does not) change system dynamics significantly.
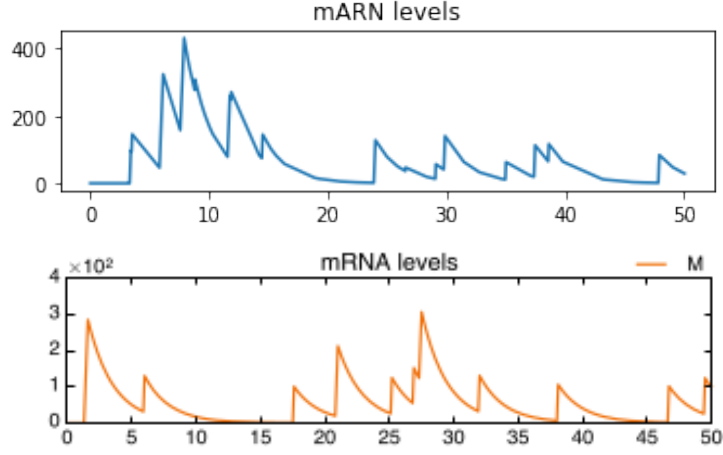
FIGURE 3 – mRNA trajectories from our model (above) and Herbach et al. (2017)'s (below) for $k_{on} = 0.34$, $k_{off} = 10$, $s_0 = 10^3$, $s_1 = 10$, $d_0 = 0.5$ and $d_1 = 0.1$ (in h^{-1})

In order to facilitate dynamical study and interpretation, I will follow Herbach et al. (2017) in spirit while devoting special attention to decouple contributions of the transcription factors and different level of dynamics.

First, I will use the same general law for the expression of k_{on} :

$$k_{on,i} = \frac{k_{0,i} + k_{1,i} \cdot \Phi_i}{1 + \Phi_i} \tag{2}$$

...where $k_{0,i}$, $k_{1,i}$ are respectively the maximal and minimal values of transition rates to the active state for gene i, and Φ_i (which I will call the "activation potential") is a mesure of the "permissivity" of the environment - that is, whether transition to the active state is favored or hampered. Intuitively, k_{on} is maximal for high Φ and minimal for low Φ, with a smooth transition around $\Phi = 1$.

However, I introduce an expression for the activation potential that accounts for all proteins multiplicatively and homogenously (while in Herbach et al. (2017) the associated protein has a special role) and includes an additive perturbation term, which I will use to force transitions in the following simulations. I also add a parameter controlling the basal activity of each gene.

$$\Phi_i = \sigma_i \cdot \prod_j \left(\frac{1 + \exp(\Theta_{i,j}) \cdot (\frac{p_j}{S_{i,j}})^{M_{i,j}}}{1 + (\frac{p_j}{S_{i,j}})^{M_{i,j}}} \right) + \delta_i \tag{3}$$

In this equation, Θ represents interaction strength, S represents the threshold for interaction, and M represents the Hill coefficient for interaction. These parameters are identical to the ones used in Herbach et al. (2017), and our Hill function also is expressed in the form $\Pi \frac{1 + a \cdot x^m}{1 + x^m}$. In this regard, our gene activation rule phenomenologically perform the same function than the original, except for the purposefully altered role of self-activation. The additional parameters are σ which expresses basal activation potential and δ which represents extrinsic perturbations.

Noting $\phi_{i,j}$ the contribution of protein j to the multiplicative term of gene i's activation function, we have :

$$\phi_{i,j}(p_j) = \frac{1 + \exp(\Theta_{i,j}) \cdot (\frac{p_j}{S_{i,j}})^{M_{i,j}}}{1 + (\frac{p_j}{S_{i,j}})^{M_{i,j}}} \tag{4}$$

This function is equivalent to a transition from 1 to $\exp(\Theta_{i,j})$ as p_j goes from 0^+ to $+\infty$. As the interaction between the $\phi_{i,j}$ terms is multiplicative, this means than gene i activation is unaffected for $p_j \to 0$ and inhibited for $p_j \xrightarrow{\Theta_{i,j}<0} +\infty$ (resp excited for $p_j \xrightarrow{\Theta_{i,j}>0} +\infty$). Moreover, the transition happens around $p_j = S_{i,j}$ and is increasingly steeper with growing $M_{i,j}$.

4

This expression allows every interaction to be modelled as a Hill function, in which we are able to control every parameter. This is useful to appreciate qualitatively the behaviour of our system.

For exemple, Φ can be approximated in the limit $M \to +\infty$ by the following expression :

$$\Phi_i \underset{M \to +\infty}{\simeq} \sigma_i \cdot \exp\left(\sum_{(j \| p_j > S_{i,j})} \Theta_{i,j}\right) + \delta_i$$

$$\text{ie} \tag{5}$$

$$\Phi_i \underset{M \to +\infty}{\simeq} \sigma_i \cdot \left(\prod_{(j \| p_j > S_{i,j})} \exp(\Theta_{i,j})\right) + \delta_i$$

This simplified expression will help us characterise the behaviour of our system while trying to build memory in a double toggle switch model.

1.3 Building the double toggle switch

We will now adress the central point of our enterprise, which is the implementation of in silico epigenetic memory in the form of a double toggle switch.

Our first task will be to restrict the parameter space, as in the model above three interacting genes correspond to a total of 51 free parameters (24 for gene-specific parameters, 27 for interaction matrixes), which is absolutely intractable for an in depth dynamic analysis.

Taking into account the structure of the desired network, we are able to remove some parameters. Indeed, a double toggle switch by definition has the structure visible figure 4.

This allows to ignore all interactions between gene 1 and 3, which removes 6 free parameters from our problem.

FIGURE 4 – Double toggle switch structure
While (1,2) and (2,3) are mutually inhibited, no interaction exist between (1,3). Self-interaction form is not specified.

We will now force as much symmetry as possible on our system. While this condition is not likely to be verified in real genetic networks, it will help greatly our purpose by weeding out parameter variations that are not key to the emergence of memory. It is likely that all existing double toggle switch obey the same dynamics as their most symmetric case (modulo rescaling), and if not the most symmetric case will be the most relevant for preliminary study anyway.

More specifically, these are the conditions we impose :
— All genes must have identical intrinsic properties, identical to those used in Herbach et al. (2017) for their toggle switch model
— Hill function coefficients must be identical for all inhibitory (resp. self-) interactions
— Threshold levels must be equal for all inhibitory (resp. self-) interactions
— Self-interaction strength must be equal for lateral genes

This reduces our parameter count from 45 to 7. Remaining parameters, and their role in interaction structure, are presented below :

$$\sigma; M = \begin{pmatrix} m_{self} & m_{lat} & 0 \\ m_{lat} & m_{self} & m_{lat} \\ 0 & m_{lat} & m_{self} \end{pmatrix} ; S = \begin{pmatrix} s_{self} & s_{lat} & +\infty \\ s_{lat} & s_{self} & s_{lat} \\ +\infty & s_{lat} & s_{self} \end{pmatrix} ; \Theta = \begin{pmatrix} \theta_{self,0} & -\theta_{lat} & 0 \\ -\dfrac{\theta_{lat}}{2} & \theta_{self,1} & -\dfrac{\theta_{lat}}{2} \\ 0 & -\theta_{lat} & \theta_{self,0} \end{pmatrix}$$

$$\tag{6}$$

All traduce critical aspects of our interaction dynamics. Therefore, we must stop here our reduction.

We will now try to find heuristics for orienting our parameter search by discretising our system to apply equation 5. Noting "+" the permissive condition of gene expression ($\phi_i << 1$) and "−" its restrictive condition ($\phi_i >> 1$), our system state can be divided into regions where activity level for each gene can either be strong ($k_{on} \sim k_{on,1}$) or weak ($k_{on} \sim k_{on,0}$). In the approximation where these regions map well onto the state space, regions can be assimilated to discrete states whose dynamical landscape is shown figure 5. Note that while this condition is verified for $M \to +\infty$, transitions zones exist between regions for $M < +\infty$ which could cause "jumps" to occur in the discretised state space.
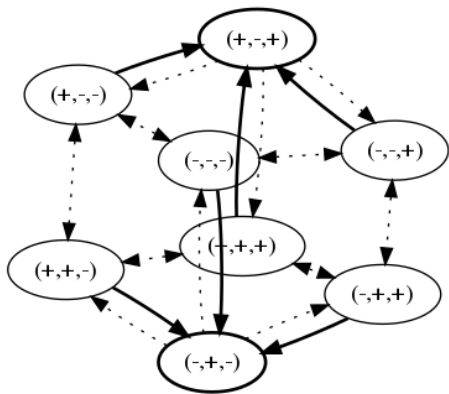

FIGURE 5 – Discretised dynamical landscape of the double toggle switch model
States coding for our memory are shown in bold
Bold lines represent possible state transitions that are useful to memory
Dotted lines represent those that are detrimental to memory

This reduction is especially relevant if, noting p^- (resp p^+) the stable typical level of a protein with its source gene in the − (resp +) condition, we have : $p^- < S_{self}, S_{lat} < p^+$, as it means that each gene expression state is informative of whether it modulates itself and its neighbours. In the limit of deterministic behaviour, steplike activation function, and steady state, we have $\phi_{i,j} = exp(\Theta_{i,j})$ if i is in state + and $\phi_{i,j} = 1$ otherwise. In other words, the double toggle switch behaves exactly as a single gene expression model with parameters imposed by equation 5 inside a given discretised state.

Transcient dynamics (ie what happens when gene activation patterns have changed but protein level not yet followed) are poorly captured in this framework, but this is of no importance for our present study as memory by definition occurs along long time scales. Functional dynamics of our double toggle switch are therefore fully accounted for in this discretised reduction. Indeed, the only dynamic internal to each region is the stochasticity intrinsic to gene expression, which is not related to interaction structure and act only as a source of noise adverse to memory. The model can therefore be divided between two scales : it acts as a deterministic boolean network at the level of the discretised states ; and as a stochastic two-state gene expression model without interaction below. Both levels can be bridged by introducing stochasticity in the transition between discrete-level states.

While the discretised model essentially closes the door for exact analytical approaches of our problem as the process is not necessarily Markovian at the discretised level (and its temporal correlations, emerging from a first passage process in an irregular space, are essentially impossible to calculate), it greatly reduces the conceptual complexity of the toggle switch system by building a relevant framework where to apply equation 5.

First, both $(+, -, +)$ and $(-, +, -)$ must be stable at the boolean level and in the absence of perturbation for the double toggle switch to perform its function. This can be traduced by the conditions $\sigma \cdot \exp(\theta) \gg 1$ for all elements of Θ.

In addition, because sub-boolean level fluctuations will routinely bring the system near the boundaries of $(+, -, +)$ and $(-, +, -)$, all other states must be unstable for the system not to derive. More precisely, they should redirect to the stable state they are most likely to have derived from, which means following the transitions that are bolded in figure 5. This allow to derive the conditions $\sigma \cdot \exp(\theta_{self} - \theta_{lat}) \ll 1$ for both θ_{self} values

Due to the fast growth of the *exp* function, these conditions can be expressed as :

$$\theta_{self,0} > -\ln(\sigma)$$
$$\theta_{self,1} > -\ln(\sigma)$$
$$\theta_{lat} > -\ln(\sigma) \tag{7}$$
$$\theta_{lat} > \theta_{self,0} + \ln(\sigma)$$
$$\theta_{lat} > \theta_{self,1} + \ln(\sigma)$$

In first approach, we take values similar to those in Herbach et al. (2017) for their toggle switch model, which respect conditions established in equation 7 : $\sigma = 1; m_{self} = 3; m_{lat} = 2; s_{self} = 1.9 \cdot 10^4; s_{lat} = 2 \cdot 10^3; \theta_{self,0} = \theta_{self,1} = 4; \theta_{lat} = 8$.

We will now test this network's property relatively to memory.

2   Characterisation of the double toggle switch's memory

Using the δ parameter, we are able to design different experimental parameters for testing our sytem's dynamics. The first test we will run is for bistability, for which I show plots figure 6. An extremely strong perturbation forces the system in the (-,+,-) state for $100h$, then removed for $400h$. The process is then repeated for the (+,-,+) state. The perturbation is visible in the $\log \Phi$ space as a steplike pattern. If a state is unstable, this will be visible as the system won't hold it during the rest time.
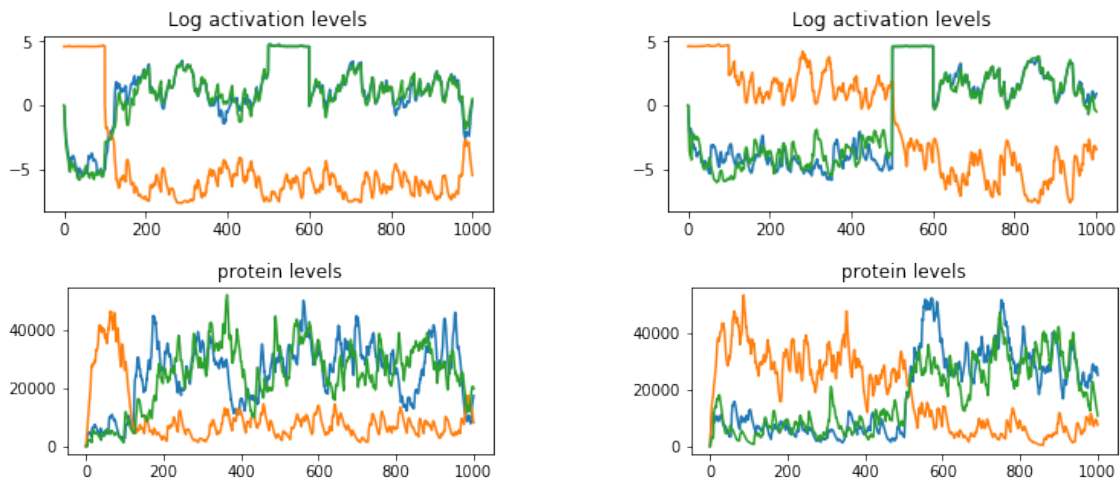

FIGURE 6 – Bistability test of the double toggle switch
Left : with Herbach et al. (2017)'s parameters
Right : with adjusted central gene self-activation

In fact, with unadjusted parameters from Herbach et al. (2017), the (-,+,-) state is indeed unstable. As in the discretised state space between genes interaction are equal, this must be due to the structural asymetry between the central gene and lateral genes. Looking at the log activity trajectory, it is visible that just after the end of stimulation the level of activity is much lower for the central gene than for lateral genes. This contrasts with the predictions of the discretised model where, by construction, all genes should have the same activation in their endogenously active state. This is not surprising as $s_{lat} < p^-$ in this parameter set, which means that even in the repressed state a gene can cause the inhibition of its neighbour. In other word, the scale separation we postulated in our discretised model does not hold.

It would be rather easy to enforce bistability by imposing scale separation (for exemple by heighening s_{lat}, and lowering $k_{on,0}$ and σ). However, I believe this would negatively affect the relevance of our present model. It would not be a surprise for anyone that a stochastic model that

has been forced to act as a boolean network with noise by an engineered scale separation indeed works as a boolean network with noise. Therefore I opted to enforce bistability by adjusting $\theta_{self,1}$, which allowed bistability to occur (near $\theta_{self,1} = 6.7$) while keeping scale blurring. Corresponding trajectories are also shown figure 6.

It is on the resulting network that we will run the following tests.

2.1 Resistance to stimuli

An important property of epigenetic memory is its ability to withstand environmental noise. In our model, environmental noise is traduced as an additive term in activity potential expression.

I have characterised the resistance to forced state transition as a function of both extrinsic stimuli strength and duration. Due to how activity potential is traduced into protein levels, both strength and duration should be high enough for forcing a transition. Unconveniently, my hardware is insufficient to characterise stimuli effect in a two-dimensional landscape. Therefore, effect of stimuli strength and stimuli duration on transition probability are studied separately.

Test for resistance to transient stimuli have been conduced with the following protocol : A strong stimuli ($\delta = 100$) was applied for $50h$ to force the system on one of its stable state, then the opposite stimuli was applied for t varying between $1h$ (near certain absence of transition) and $25h$ (near certain transition). The process was repeated $n = 20$ times for each transition (($+,-,+) \rightarrow (-,+,-)$ and $(-,+,-) \rightarrow (+,-,+)$), and checking for state change in each run allowed to plot probability of state change as a function of stimuli duration for both transitions. A similar protocol was followed with varying stimuli strength for $t = 50h$ and $n = 10$.
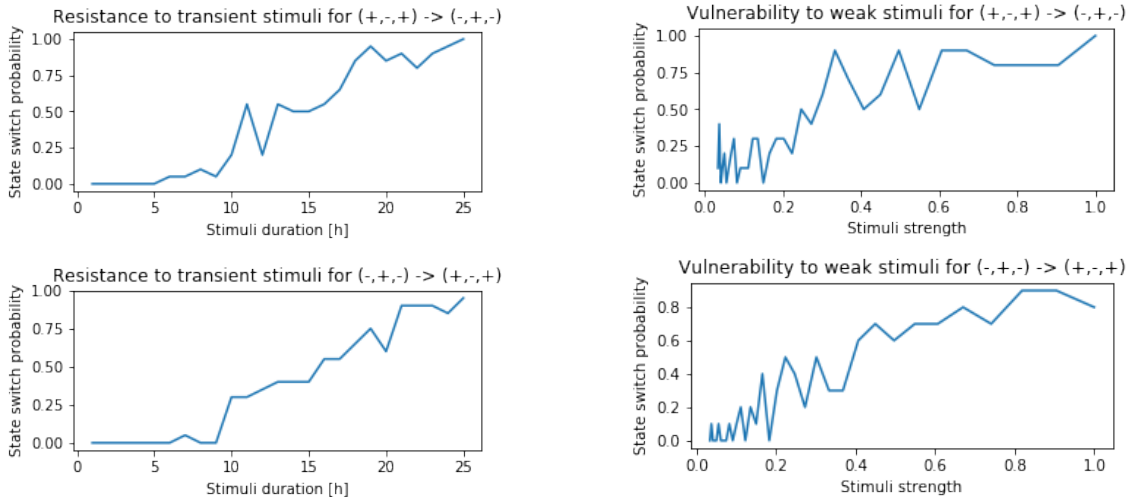

FIGURE 7 – Test for resistance to stimuli
Left : varying stimuli time with $\delta = 100$
Right : varying stimuli strength with $t = 50h$

Results are displayed figure 7. They confirm our initial conjecture : while stimuli of significant strength and duration will near certainly cause a state change, a stimuli that lack either strength or duration will near certainly have no effect on the system. Transition seem to happen around $\delta = 0.5 \pm 0.3; t = 15 \pm 5h$.

This test confirm the ability for our double toggle switch to filter out environmental noise, even in the absence of scale separation.

2.2 Equilibration

Due to the structural asymetry, one might wonder whether both the $(-,+,-)$ and $(+,-,+)$ states have similar ability to retain information. To check this, I imposed a periodic stimuli forcing
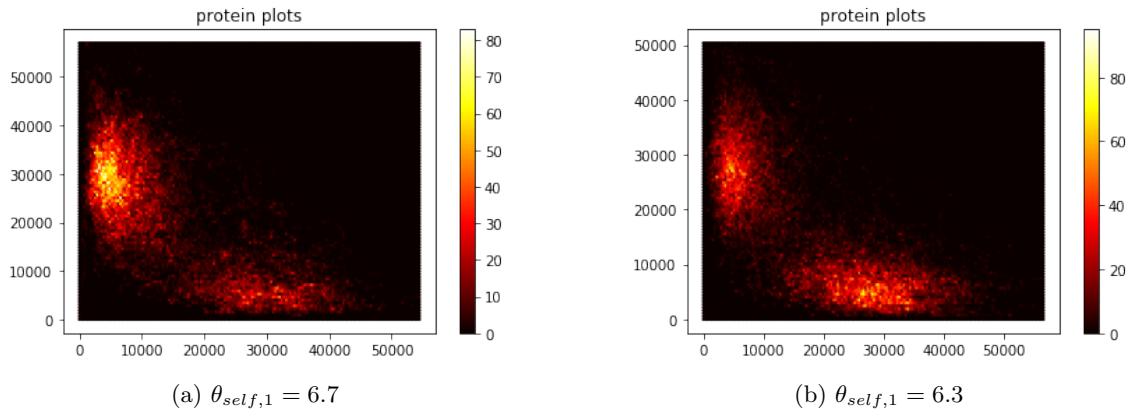
(a) $\theta_{self,1} = 6.7$

(b) $\theta_{self,1} = 6.3$

FIGURE 8 – Equilibration test
Left : with original $\theta_{self,1}$
Right : with adjusted $\theta_{self,1}$

the $(+,-,+)$ and the $(-,+,-)$ state alternatively, before letting the system evolve endogenously for $t = 1000$. Then, I plotted figure 8 a heatmap of the protein level corresponding to the central and one lateral gene during this time.

With the initial parameter of $\theta_{self,1} = 6.7$, the central gene clearly was overexpressed relatively to the lateral one. This was however easy to fix by setting $\theta_{self,1} = 6.3$.

This shows that while equilibrated balance isn't a necessary condition of the double toggle switch, it can easily be adjusted through evolution.

2.3 Memory dimensionality

On the heatmap figure 8, the system seem to simply oscillate between two attractors, as its function requires. I plotted figure 9 additional graphics to check whether it actually is the case, or additional functional dynamics do exist beside the 1-bit memory.

As the activation potential seem to closely follow a oblique line in log space (vertical and horizontal lines correspond to artificial stimulation) with a clear separation near $\log(\Phi) = -1$, it seems likely that no information other than the discrete-level state is informative of activation dynamics.

In other word, beside the binary memory we purposely implemented in our system, all existing dynamics can be understood as noise.
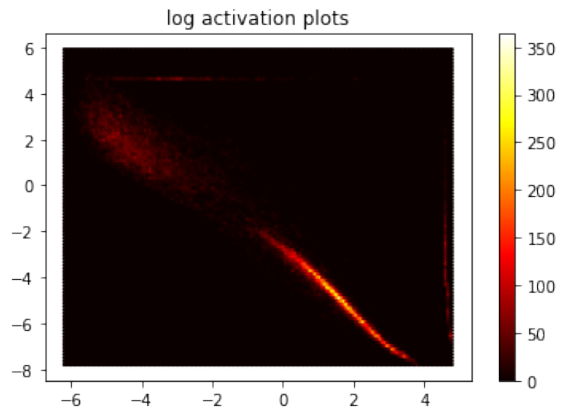

FIGURE 9 – Memory dimensionality test
$\log(\Phi)$ for the central and one lateral gene

9

Conclusion

In the present work, I built a model of the double toggle switch motif in the context of genetic regulation network and analysed its functional and dynamic properties.

I argued that, in the model I studied, the network could act as a boolean network with noise in the context of "scale separation" (that is, when repressed genes never influence themselve or their neighbour, and active genes always influence them at maximum level) as it allows to introduce a binary reduction of the system statespace that traduces all functional dynamics. I studied computationally a case where scale separation is not respected, and showed that it could still retain information in the context of irrelevant (weak or transient) stimuli, and that system dynamics can be described as a binary memory with noise.

Open questions still exist that are beyond the scope of this study. For exemple, by plotting the distribution of endogenous state switch as a function of run time, we could check whether the process can empirically be described as Markovian at the discretised states level. In this case, the double toggle switch could be functionally characterised as a simple two-state Markov process, with change rates depending on endogenous parameters and exogenous stimuli.

This would allow straightforward analytic resolution of the system's functional properties (that is, of the dynamics of its memory), without information loss. While it is likely verified in the case of scale separation, it is ambiguous whether the Markov property still holds without a clear definition of the discretised states it would model, and whether these temporal correlation can be accounted for in a model based on stochastic transition between discrete quasi stable states.

In any case, the study of endogenous behaviour of gene regulation networks is critical to developmental biology. Functional models of simple motifs might help in identifying emerging organism-level properties relevant to evolution or medicine. I hope these approaches one day allow similar advances to those discussed in introduction.

Références

Barabási, A.-L. and Bonabeau, E. (2003). Scale-free networks. *Scientific american*, 288(5) :60–69.

Herbach, U., Bonnaffoux, A., Espinasse, T., and Gandrillon, O. (2017). Inferring gene regulatory networks from single-cell data : a mechanistic approach. *BMC systems biology*, 11(1) :105.

Huang, J. and Ulanowicz, R. E. (2014). Ecological network analysis for economic systems : growth and development and implications for sustainable development. *PloS one*, 9(6) :e100923.

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D., and Alon, U. (2002). Network motifs : simple building blocks of complex networks. *Science*, 298(5594) :824–827.

Mizeranschi, A., Zheng, H., Thompson, P., and Dubitzky, W. (2015). Evaluating a common semi-mechanistic mathematical model of gene-regulatory networks. *BMC systems biology*, 9(5) :S2.

Munsky, B., Neuert, G., and Van Oudenaarden, A. (2012). Using gene expression noise to understand gene regulation. *Science*, 336(6078) :183–187.

Ulanowicz, R. E. (2004). Quantitative methods for ecological network analysis. *Computational Biology and Chemistry*, 28(5-6) :321–339.

Viñuelas, J., Kaneko, G., Coulon, A., Vallin, E., Morin, V., Mejia-Pous, C., Kupiec, J.-J., Beslon, G., and Gandrillon, O. (2013). Quantifying the contribution of chromatin dynamics to stochastic gene expression reveals long, locus-dependent periods between transcriptional bursts. *BMC biology*, 11(1) :15.

Von Dassow, G., Meir, E., Munro, E. M., and Odell, G. M. (2000). The segment polarity network is a robust developmental module. *Nature*, 406(6792) :188.

Wagner, G. P., Pavlicev, M., and Cheverud, J. M. (2007). The road to modularity. *Nature Reviews Genetics*, 8(12) :921.