REDUCTION OF A STOCHASTIC MODEL OF GENE EXPRESSION: LAGRANGIAN DYNAMICS GIVES ACCESS TO BASINS OF ATTRACTION AS CELL TYPES AND METASTABILTY

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Abstract: Differentiation is the process whereby a cell acquires a specific phenotype, by differential gene expression as a function of time. This is thought to result from the dynamical functioning of an underlying Gene Regulatory Network (GRN). The precise path from the stochastic GRN behavior to the resulting cell state is still an open question. In this work we propose to reduce a stochastic model of gene expression, where a cell is represented by a vector in a continuous space of gene expression, to a discrete coarse-grained model on a limited number of cell types. We develop analytical results and numerical tools to perform this reduction for a specific model characterizing the evolution of a cell by a system of piecewise deterministic Markov processes (PDMP). Solving a spectral problem, we find the explicit variational form of the rate function associated to a Large deviations principle, for any number of genes. The resulting Lagrangian dynamics allows us to define a deterministic limit, the basins of attraction of which can be identified to cellular types. In this context the quasipotential, describing the transitions between these basins in the weak noise limit, can be defined as the unique solution of an Hamilton-Jacobi equation under a particular constraint. We develop a numerical method for approximating the coarse-grained model parameters, and show its accuracy for a symmetric toggle-switch network. We deduce from the reduced model an analytical approximation of the stationary distribution of the PDMP system, which appears as a Beta mixture. Altogether those results establish a rigorous frame for connecting GRN behavior to the resulting cellular behavior, including the calculation of the probability of jumps between cell types.

Keywords: Single cell, Gene Regulation Network, Energetic Landscape, Piecewise Deterministic Markov Processes, Large deviation, Metastability

Introduction

Differentiation is the process whereby a cell acquires a specific phenotype, by differential gene expression as a function of time. Measuring how gene expression changes as differentiation proceeds is therefore of essence to understand this process. Advances in measurement technologies now allow to obtain gene expression levels at the single cell level. It offers a much more accurate view than population-based measurements, that has been obscured by mean population-based averaging [1], [2]. It has among other established that there is a high cell-to-cell variability in gene expression, and that this variability has to be taken into account when examining a differentiation process at the single-cell level [3], [4], [5], [6], [7], [8], [9], [10],[11].
A popular vision of the cellular evolution during differentiation, introduced by Waddington in [12], is to compare cells to marbles following probabilistic trajectories, as they roll through a developmental landscape of ridges and valleys. These trajectories are represented in the gene expression space: a cell can be described by a vector, each coordinate of which represents the expression of a gene [13], [14]. Thus, the state of a cell is characterized by its position in the gene expression space, i.e its specific level for all of its expressed genes. This landscape is often regarded to be shaped by the underlying gene regulatory network (GRN), the behavior of which can be influenced by many factors, such as proliferation or cell-to-cell communication.
A cell has theoretically as many states as the combination of proteins quantity possibly associated to each gene, which is potentially huge [15]. But metastability seems inherent to cell differentiation processes, as evidenced by limited number of existing cellular phenotypes [16], [17], providing a rationale for dimension reduction approaches [18]. Indeed, since [19] and [20], many authors have identified cell types with the basins of attraction of a dynamical system modeling the differentiation process, although the very concept of "cell type" has to be interrogated in the era of single-cell omics [21].
Adapting this identification for characterizing metastability in the case of stochastic models of gene expression has been studied mostly in the context of stochastic diffusion [22], [23], [24], but also for stochastic hybrid systems [25]. In the weak noise limit, a natural development of this analysis consists in describing the transitions between different macrostates within the Large deviations framework [26], [27].
We are going to apply this strategy for a piecewise-deterministic Markov process (PDMP) describing GRN dynamics within a single cell, introduced in [28], which corresponds accurately to the non-Gaussian distribution of single–cell gene expression data. The main contribution of this article is to derive the explicit variational form of the rate function associated to a Large deviations principle (LDP) for this model. We replace this result in the context of studying metastability, and discuss the conditions of existence and uniqueness of a quasipotential able to describe transitions between basins. For any network, we provide a numerical method for computing these transition times, and deduce a non-Gaussian mixture model able to approximate proteins distribution.

1 Materials and methods

1.1 GRN model and fast transcription reduction

We recall briefly the PDMP model, which is described in details in [28], based on a hybrid version of the well-established two-state model of gene expression [29], [30] including both mRNA and protein production [31] and illustrated in Figure 1.
A gene is described by the state of a promoter, which can be $\{on, off\}$. If the promoter is on, mRNAs will be transcripted with a rate s_0 and degraded with a rate d_0. If it is off, only mRNA degradation occurs. Translation of mRNAs into proteins happens regardless of the promoter state at a rate s_1, and protein degradation at a rate d_1. Neglecting the molecular noise of proteins and mRNAs, we obtain the hybrid model:

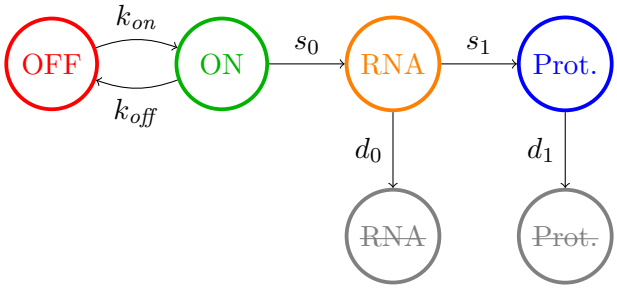


Figure 1: The two-states model of gene expression.
Ref: [30], [28]

$$\begin{cases} E(t) : 0 \xrightarrow{k_{on}} 1, 1 \xrightarrow{k_{off}} 0, \\ M'(t) = s_0 E(t) - d_0 M(t), \\ P'(t) = s_1 M(t) - d_1 P(t). \end{cases}$$

where $(E(t), M(t), P(t))$ denote respectively the promoter, mRNA and protein concentration at time t.
The key idea is to put this two-states model in a network. Denoting n the number of genes, the vector (E, M, P) describing the process is now of dimension $3n$. The jump rate of each gene i is characterized by two specific functions $k_{on,i}$ and $k_{off,i}$.
To take into account the interactions between genes, we consider that for all $i = 1, \cdots, n$, $k_{on,i}$ is a function of the full vector P lower bounded by positive constant. The function is chosen such that if gene i activates gene j, then $\partial_{P_i} k_{on,j} \geq 0$. For the sake of simplicity, we consider that $k_{off,i}$ does not depend on the protein level. Considering the bursting nature of mRNA, we assume $k_{off,i} \gg k_{on,i}$.
Two modifications are performed in [28] to this mechanistic model. First, the parameters s_0 and s_1 can be removed to obtain a dimensionless model, from which physical trajectories can be retrieved with a simple rescaling.
Second, a scaling analysis leads to simplify the model. Indeed, degradation rates plays a crucial role in the dynamics of the system. The ratio $\frac{d_{0,i}}{d_{1,i}}$ controls the buffering of promoter noise by mRNAs and, since $k_{off,i} \gg k_{on,i}$, the ratio $\frac{k_{on,i}}{d_{0,i}}$ controls the buffering of mRNA noise by proteins. It is often considered that promoter switches and mRNA bursts are fast in regard to protein dynamics, i.e $\frac{d_{0,i}}{d_{1,i}} \gg 1$ with $\frac{k_{on,i}}{d_{0,i}}$ fixed. The correlation between mRNAs and proteins produced by the gene is then very small, and the model can be reduced by removing mRNA and making proteins directly depend on the promoters. The value of the ratio $\frac{d_{0,i}}{d_{1,i}}$ is evaluated around 5 [32].
Considering the mean value of the function $k_{on,i}$, denoted $\overline{k_i}$, which corresponds to its value when the gene i does no interact with the GRN, we can also take the protein timescale as a reference by fixing $d_{1,i}$, and rescale the interaction functions with the coefficient $\varepsilon = \frac{d_{1,i}}{\overline{k_i}}$. Assuming that $\frac{\overline{k_i}}{d_{0,i}}$ is greater than 1, ε is smaller than 1/5. We then replace $(k_{on,i}, k_{off,i}) \leftarrow (\frac{\tilde{k}_{on,i}}{\varepsilon}, \frac{\tilde{k}_{off,i}}{\varepsilon})$. For the sake of simplicity, we will write from now, $k_{on,i}$ and $k_{off,i}$ instead of $\tilde{k}_{on,i}$ and $\tilde{k}_{off,i}$.

We obtain a reduced dimensionless system for any network of n genes:

$$\forall i \in \{1, \cdots, n\} : \begin{cases} E_i(t) : 0 \xrightarrow{\frac{k_{on,i}(X(t))}{\varepsilon}} 1, 1 \xrightarrow{\frac{k_{off,i}}{\varepsilon}} 0, \\ X_i'(t) = d_i(E_i(t) - X_i(t)). \end{cases} \tag{1}$$

Here, X describes the protein vector in the renormalized gene expression space $\Omega := (0, 1)^n$ and

E describes the promoter state, in $S_e := \{0,1\}^n$, where we identify the state *off* with 0, and the state *on* with 1.
As $\text{card}(S_E) = 2^n$, we can write the joint probability density $u(t,e,x)$ of (E_t, X_t) as a 2^n-dimensional vector $u(t,x) = u_e(t,x)_{e\in S_E} \in \mathbb{R}^{2^n}$. The master equation on u can be written:

$$\frac{\partial u}{\partial t}(t,x) + \sum_{i=1}^{n} \frac{\partial}{\partial x_i}\left(F_i(x)u(t,x)\right) = \frac{1}{\varepsilon}\sum_{i=1}^{n} K_i(x)u(t,x). \tag{2}$$

For all $i = 1,\cdots,n$, for all $x \in \Omega$, $F_i(x)$ and $K_i(x)$ are matrices of size 2^n. Each F_i is diagonal, and the term on a line associated to a promoter state e corresponds to the drift of gene i: $d_i(e_i - x_i)$. K_i is not diagonal: each state e is coupled with every state e' such that only the coordinate e_i changes in e, from 1 to 0 or conversely. Each of these matrices can be expressed as a tensorial product of $(n-1)$ two-dimensional identity matrices with a two-dimensional matrix corresponding to the operator associated to an isolated gene.

- $F_i(x) = I_2 \otimes \cdots \otimes \underbrace{F^{(i)}(x)}_{\text{i-th position}} \otimes \cdots \otimes I_2$
- $K_i(x) = I_2 \otimes \cdots \otimes \underbrace{K^{(i)}(x)}_{\text{i-th position}} \otimes \cdots \otimes I_2,$
- $F^{(i)}(x) = \begin{pmatrix} -d_i x_i & 0 \\ 0 & d_i(1-x_i) \end{pmatrix}$
- $K^{(i)}(x) = \begin{pmatrix} -k_{on,i}(x) & k_{off,i}(x) \\ k_{on,i}(x) & -k_{off,i}(x) \end{pmatrix}.$

We detail in Appendix A the two-dimensional case for a better understanding of this tensorial expression.

1.2 Deterministic approximation in the weak noise limit

The previous model describes the promoter state of every gene i at each time as a Bernoulli random variable. We use the biological fact that promoter switches are frequent in regard to protein dynamic, i.e $\varepsilon < 1$ with the previous notations. When $\varepsilon \ll 1$, we can approximate the conditional distribution of the promoters knowing proteins, ρ, by its quasistationary approximation $\overline{\rho}$:

$$\forall i = 1,\cdots,n,\ \forall x \in \Omega :\ \rho_i(x) \simeq \overline{\rho}_i(x) = \frac{k_{on,i}(x)}{k_{off,i} + k_{on,i}(x)} \tag{3}$$

which is derived from the stationary distribution of the Markov chain on the promoters states for a given value of the protein vector $X = x$, defined by the matrix $\sum_{i=1}^{n} K_i(x)$ (see [33], [34]).
Thus, the PDMP model (1) can be coarsely approximated by a system of ordinary differential equations:

$$\forall i = 1,\cdots,n :\ \dot{x}_i(t) = d_i\left(\frac{k_{on,i}(x(t))}{k_{off,i} + k_{on,i}(x(t))} - x_i(t)\right). \tag{4}$$

Intuitively, these trajectories correspond to the mean behaviour of a cell in the weak noise limit, i.e when promoters jump much faster than proteins concentration changes. We will show in Section 2.4 that for any $T < \infty$, a random path $(X^\epsilon(t))_{0\le t\le T}$ converges in probability to a trajectory $(x(t))_{0\le t\le T}$ solution of the system (4) when $\varepsilon \to 0$. The diffusion limit, which keeps a residual noise scaled by $\sqrt{\varepsilon}$, can also be rigorously derived from the PDMP system [35].

Even if this strongly depends on the interaction functions $(k_{on,i})_{i=1,\cdots,n}$ and model parameters, we assume that any limit set of a trajectory solution of the system (4) as $t \to +\infty$ is reduced to a single point, described by one of the solutions of:

$$\forall i = 1,\cdots,n :\ \frac{k_{on,i}(x)}{k_{off,i} + k_{on,i}(x)} - x_i = 0. \tag{5}$$

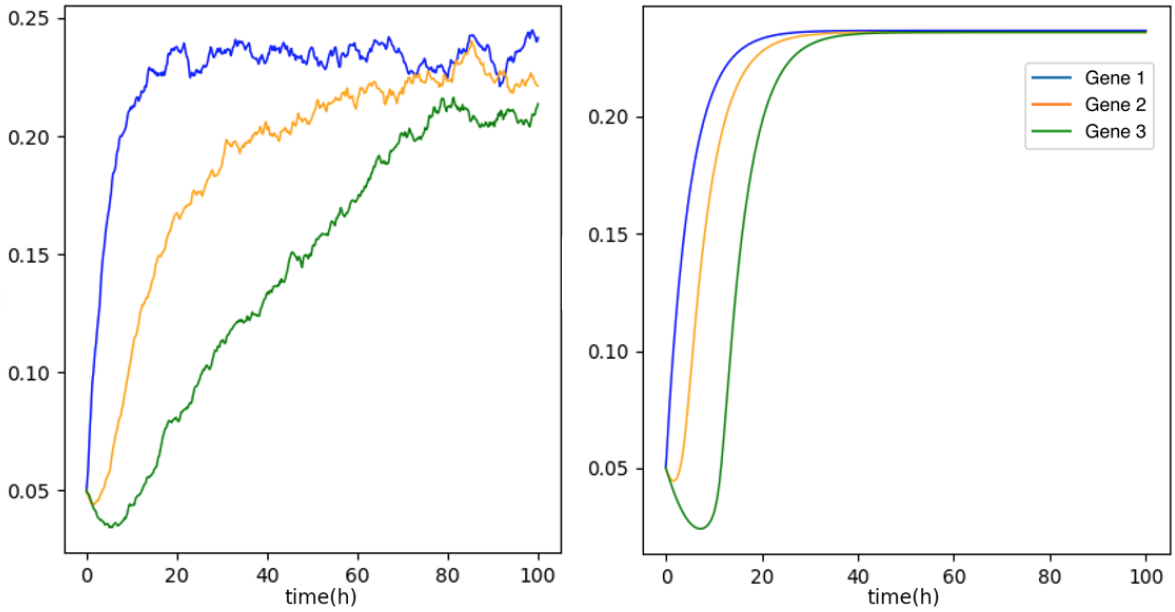


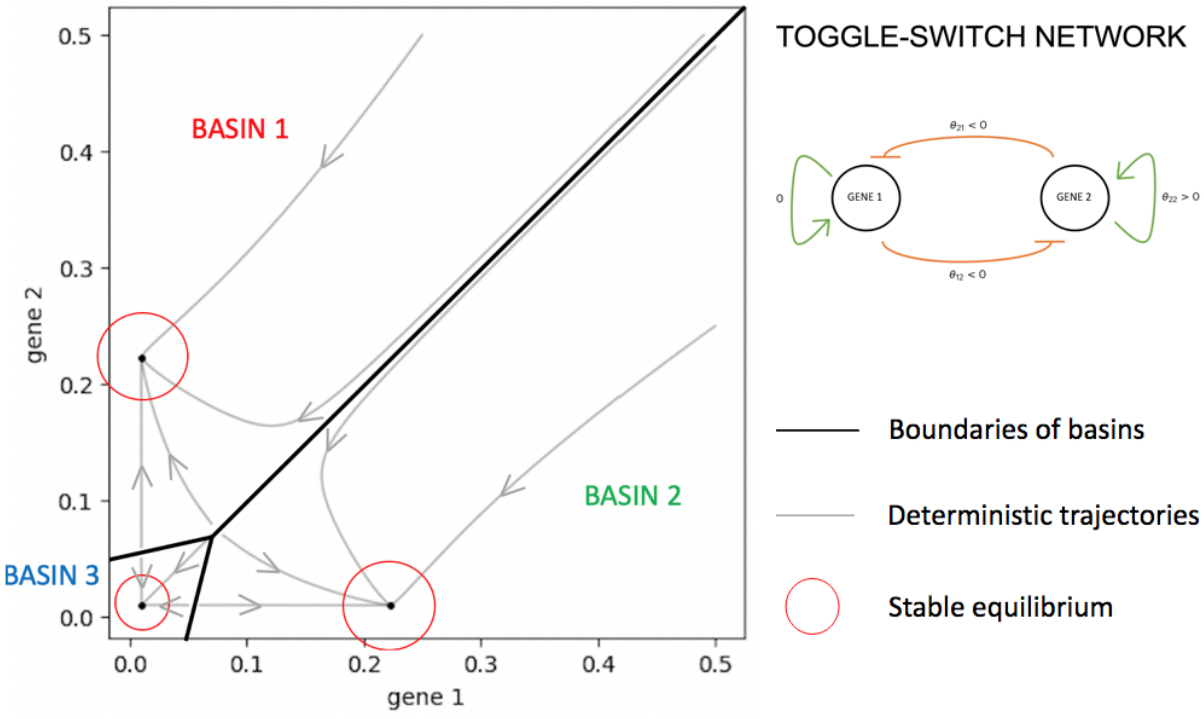
Figure 2: Comparison between the average on a large number of simulated trajectories (shown on the left) with $\varepsilon = 1/7$ and the trajectories generated by the deterministic system (shown on the right) for a single pathway network: gene 1 $\longrightarrow$ gene 2 $\longrightarrow$ gene 3.

Alternatively speaking, we rule out the existence of attractive limit cycles or more complicated orbits. The gene expression space Ω can be then decomposed in many basins of attraction, associated to the attractors of the system (5).

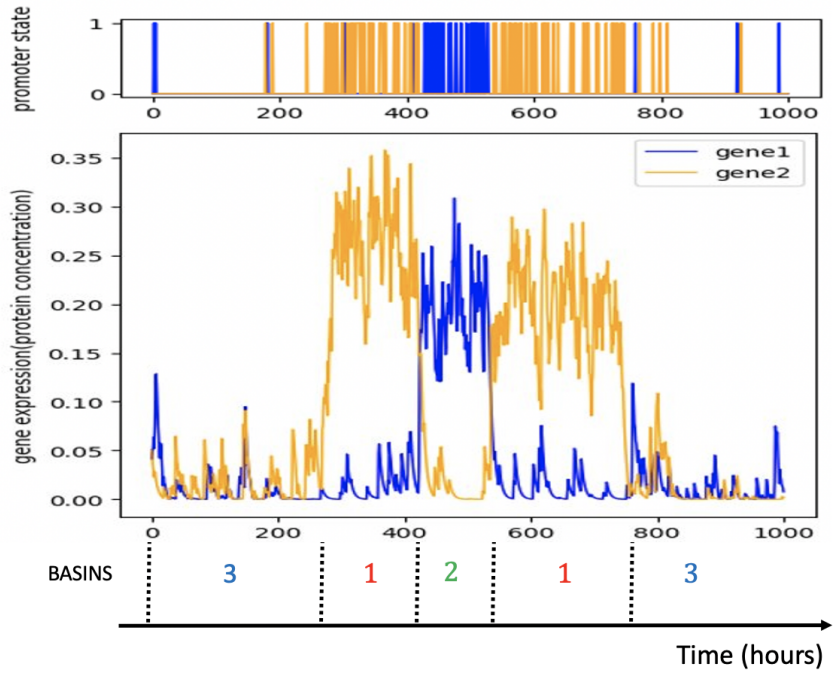
Without noise, the fate of a cell trajectory is fully characterized by its initial state x_0. Generically, it converges to the attractor of the basin of attraction it belongs to, which is a single point by assumption.
Noise can modify the deterministic trajectories in at least two ways. In short time, a stochastic trajectory can deviate significantly from the deterministic one, which is expected for biologically relevant parameters. In the case of a single, global, attractor, the deterministic system allows to retrieve the global dynamics of the process, i.e the equilibrium and the order of convergence between the different genes (see Figure 2).
In long time, stochastic dynamics can even push the trajectory out of its basin of attraction to another one, changing radically the fate of the cell. These transitions cannot be catched by the deterministic limit, and happen on a time scale which is expected to be of the order of $e^{\frac{C}{\varepsilon}}$ (owing to a Large deviations principle studied below), where C is an unknown constant depending on the basins. We illustrate this situation for a toggle-switch network, which generates complex behaviours in two dimensions (see Figure 3a). An example of random path, the stochastic evolution of promoters and proteins along time, is represented in Figure 3b. All the details on the interaction functions and the parameters used for this network can be found respectively in the Appendices B and C.

1.3 Metastability

For a small ε, the passage of a single cell from one basin of attraction to another is a rare event: the process will spend in each basin a time long enough to equilibrate inside.
Adopting the paradigm of metastability referred in the introduction, we identify each cell type to a basin of attraction associated to a stable equilibrium of the deterministic system (4). In this point of view, a cell type corresponds to a metastable sub-region of the gene expression



(a)



(b)

Figure 3: 3a : Phase portrait of the deterministic approximation for a symmetric toggle-switch with strong inhibition: two genes which activate themselves and inhibit each other.
3b : Example of a stochastic trajectory generated by the toggle-switch, for $\varepsilon = 1/8$.

space. It also corresponds to the notion of macrostate used in the theory of Markov State Models which has been recently applied to the study of a discrete cell differentiation model in [36]. Provided we can describe accurately the rates of transition between the basins on the long run, the process can then be coarsely reduced to a new discrete process on the cell types.

More precisely, let m be the number of basins of attraction of the system (4). We denote Z the set of these m basins, that we order arbitrarily: $Z = \{Z_1, \cdots, Z_m\}$. The stable equilibrium points of the system (5), called attractors are denoted by $(X_{eq,Z_i})_{Z_i \in Z}$. Each attractor is associated to a specific basin of attraction. The closure of these m basins, $(\overline{Z}_i)_{i\in\{1,\cdots,m\}}$, covers the gene expression space Ω. For characterizing explicitly metastability, we are going to build a discrete process $(\hat{Z}_l^\varepsilon)_{l\in\mathbb{N}}$, with values in Z. From a random path X_t^ε of the PDMP system such that $X_0^\varepsilon \in Z$, we define the discrete process $\hat{Z}_l^\varepsilon$, which describes the cell types, as follows:

$$\forall l \in \mathbb{N} : \hat{Z}_l^\varepsilon = \sum_{i=1}^{m} Z_i \mathbb{1}_{\{X_{\tau_l}^\varepsilon \in Z_i\}},$$

where $(\tau_l)_{l\in\mathbb{N}}$ is a sequence of stopping times defined by:

$$\begin{aligned} \tau_0^\varepsilon &= 0, \\ \forall l \in \mathbb{N}^* : \tau_l^\varepsilon &= \inf\{t \geq \tau_{l-1}^\varepsilon \mid X_t^\varepsilon \in Z \setminus \hat{Z}_{l-1}^\varepsilon\}, \end{aligned}$$

As we will see in Section 2.4 with more details, for every basin Z_i such that $\hat{Z}_l = Z_i$, whatever is the point on the boundary of Z_i which has been first attained, X_t^ε reaches the attractor X_{eq,Z_i} of Z_i before leaving the basin, with probability converging to 1 as $\varepsilon \to 0$. Thus, for any basin Z_j, the probability $\mathbb{P}(\hat{Z}_{l+1} = Z_j)$ is asymptotically entirely characterized by the value of $\hat{Z}_l$, as it is asymptotically independent of the trajectory converging to X_{eq,Z_i} and, in particular, of the value of $X_{\tau_l}^\varepsilon$. In other words, $(\hat{Z}_l)$ converges to a Markov chain when $\varepsilon \to 0$. For small ε, it is natural to approximate the distribution of the exit time from a basin by an exponential distribution. The *in silico* distribution represented in Figure 4 illustrates this assumption, even if the exponential distribution seems to overestimate the smallest values.

To completely characterize metastability, it remains to compute the rates of these exponential transition laws $\{a_{ij}^\varepsilon\}_{i,j}$, defined $\forall Z_i, Z_j \in Z^2$, $i \neq j$, by:

$$a_{ij}^\varepsilon = \frac{1}{\overline{T}_{Z_i,Z_j}^\varepsilon},$$

where $\overline{T}_{Z_i,Z_j}^\varepsilon = \mathbb{E}(\tau_1^\varepsilon \mid \hat{Z}_1^\varepsilon = Z_j, \hat{Z}_0^\varepsilon = Z_i)$ is the Mean First Passage Time (MFPT) of exit from Z_i to Z_j. These transition rates characterize a time-continuous Markov process Z_t^ε, with discrete state space Z. It represents accurately, when ε is small enough, the main dynamics of the system. This reduced model is then fully described by m^2 transition rates: when the number of genes n is large, it is significantly smaller than the n^2 parameters characterizing the GRN model (see Appendix B).

1.4 MFPTs' numerical approximation

The collection of MFPTs described previously are conditional expectations, conditioned on rare events: when $\varepsilon \ll 1$ or when the number of genes is large, they would be impossible to compute with a crude Monte-Carlo method.
In this Section, we justify that a numerical method based on a splitting algorithm allows to compute the MFPTs between any couple of basins (Z_i,Z_j), $j \neq i$.

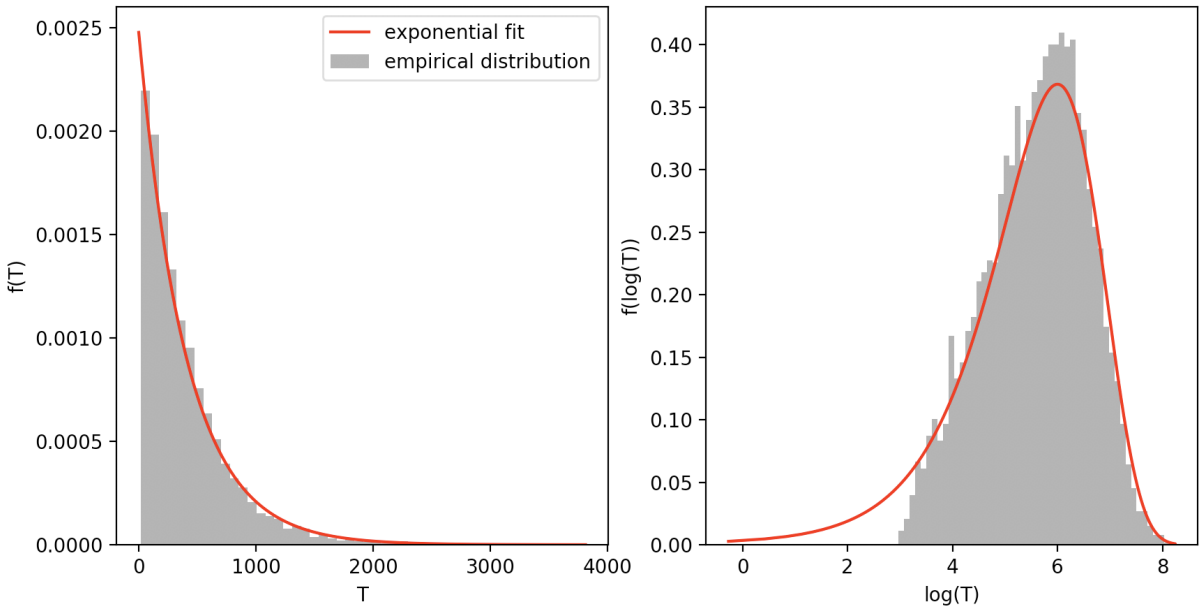


Figure 4: Comparison between the distribution of the exit time from a basin, obtained with a Monte-Carlo method, and the exponential law with appropriate expected value, for $\varepsilon = 1/8$. We represent the two densities in normal scale (on the left-hand side) and in logarithmic scale (on the right-hand side) to observe the overestimation of smallest values.

1.4.1 General setting

Let consider $R > 0$, and $r < R$, two small parameters characterizing two small neighborhoods of the attractor of Z_i: we denote γ_{Z_i} the r-neighborhood of X_{eq,Z_i}, and Γ_{Z_i} its R-neighborhood. Splitting algorithms can provide an unbiased estimator of the probability, for a random path X_t^ε of the PDMP system starting in $x \in Z_i$, of reaching a basin Z_j before entering in γ_{Z_i} :

$$p_{ij}^\varepsilon(x) = \mathbb{P}_x^\varepsilon \left(\tau_{Z_j}^\varepsilon < \tau_{\gamma_{Z_i} \cup \{Z \setminus \{\overline{Z_i} \cup \overline{Z_j}\}\}}^\varepsilon \right), \tag{6}$$

where $x \in Z_i$ and $\tau_A^\varepsilon = \inf\{t \geq 0 \mid X_0^\varepsilon = x, X_t^\varepsilon \in A\}$, $\forall A \subset \Omega$.

To obtain an approximation of $\overline{T}_{Z_i,Z_j}^\varepsilon$ from the probability (6), we follow a method of [37] which consists in cutting a transition path into two pieces: a piece going from X_{eq,Z_i} to $\partial\Gamma_{Z_i}$, and another reaching Z_j from $\partial\Gamma_{Z_i}$.
Consider the random path X_t^ε generated by the PDMP, with initial condition $X_0^\varepsilon \in Z_i$. We define the stopping times $(\mu_1^\varepsilon, \cdots, \mu_l^\varepsilon), (\sigma_1^\varepsilon, \cdots, \sigma_l^\varepsilon)$ defined by: $\mu_0^\varepsilon = 0$, $\forall l \in \mathbb{N}$,
$\sigma_l^\varepsilon = \inf\{t \geq \mu_l^\varepsilon \mid X_t^\varepsilon \in \{\gamma_{Z_i} \cup \{Z \setminus \{\overline{Z_i} \cup \overline{Z_j}\}\}\} \cup \partial Z_j\}$ and $\mu_{l+1}^\varepsilon = \inf\{t \geq \sigma_l^\varepsilon \mid X_t^\varepsilon \in Z_i \setminus \Gamma_{Z_i}\}$ (see Figure 5).
We define the chain $Y_l^\varepsilon = X_{\sigma_l}^\varepsilon$. If $Y_l^\varepsilon \in \partial Z_j$, we set $\forall k > l : \sigma_k^\varepsilon = \mu_k^\varepsilon = \infty$ and the chain Y_l stops.

1.4.2 Method for one gene

Consider the case of one gene: the boundary set $\partial\Gamma_{Z_i}$ is reduced to a single point x_0. Thus, in such one-dimensional gene expression space, a random path crosses x_0 to both exit Γ_{Z_i} and come back to γ_{Z_i} if it does not leave the basin before: the Markov property of the PDMP process then justifies that the quantities $\mathbb{E}(\mu_l^\varepsilon - \sigma_{l-1}^\varepsilon)$ and $\mathbb{E}(\sigma_l^\varepsilon - \mu_l^\varepsilon \mid Y_l^\varepsilon \notin \partial Z_j)$ do not depend on l.
As explained in [37], the random variable $W_{ij}^\varepsilon = \inf\{l \mid Y_0^\varepsilon = x_0, Y_l^\varepsilon \in \partial Z_j\}$ follows then a

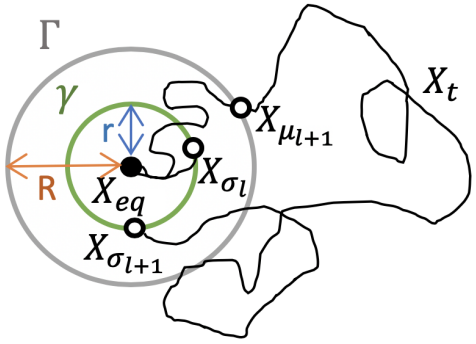


Figure 5: Illustration for a random path X_t of the stopping times describing the entrance in a r-neighborhood γ (σ) and the exit from a R-neighborhood Γ (μ) of an attractor X_{eq}.

geometric distribution, with expected value $(p_{ij}^{\varepsilon}(x_0))^{-1}$.
Let denote $\overline{T}^{\varepsilon}_{Z_i,Z_i} = \mathbb{E}(\sigma_{l+1}^{\varepsilon} - \sigma_l^{\varepsilon} \mid \forall j, Y_l^{\varepsilon} \notin \partial Z_j)$. The MFPT $\overline{T}^{\varepsilon}_{Z_i,Z_j}$ is equal to the mean number of attempts of exit from Z_i without reaching γ_{Z_i}, multiplied by the mean time of the trajectories which do not reach ∂Z_j, added to the mean time to reach γ_{Z_i} for the first time from any point of Z_i, and to the mean time to reach ∂Z_j from the first time from any point of $\partial\Gamma_{Z_i}$. These two last quantities are negligible with respect to the first one when the event of interest is rare enough. Thus, for one gene and when $\varepsilon \ll 1$, the MFPT between the two basins can be approximated by the following expression:

$$\overline{T}^{\varepsilon}_{Z_i,Z_j} \simeq \frac{\overline{T}^{\varepsilon}_{Z_i,Z_i}}{p_{ij}^{\varepsilon}(x_0)}. \tag{7}$$

1.4.3 Generalization for more than one gene

The difficulty for generalizing this reasoning in more than one dimension is to keep the Markovian properties which had been used to cut the trajectories into pieces.
Heuristically, the same argument which led us to approximate the PDMP system by a Markov jump process can be used to justify the asymptotic independence on l of the quantity $\mathbb{E}(\mu_l^{\varepsilon} - \sigma_{l-1}^{\varepsilon})$: for $\varepsilon \ll 1$, any trajectory starting from $\partial\Gamma_{Z_i}$ will rapidly loose the memory of its starting point after a mixing time within Γ_{Z_i}.
But it is more complicated to conclude on the independence on l of the quantity $\mathbb{E}(\sigma_l^{\varepsilon} - \mu_l^{\varepsilon} \mid Y_l^{\varepsilon} \notin \partial Z_j)$, which strongly depends on the position of Y_l^{ε} when the gene expression space is multidimensional.
Following the proposition 1. of [37], we introduce two hypersurfaces $\gamma_{min,Z_i} = \{x \in Z_i, p_{ij}^{\varepsilon}(x) = c_1\}$ and $\Gamma_{min,Z_i} = \{x \in Z_i, p_{ij}^{\varepsilon}(x) = c_2\}$, where $c_1 < c_2$ are two constants, and we replace γ_{Z_i} and Γ_{Z_i} by γ_{min,Z_i} and Γ_{min,Z_i} in the definitions of the stopping times μ_l^{ε} and σ_l^{ε}. Intuitively, we substitute to the squared euclidean distance, used for characterizing the neighborhood γ_{Z_i} and Γ_{Z_i}, a new function based on the probability of reaching the (unknown) boundary: $\forall x, y \in Z_i$, $|| x - y ||^2 \leftarrow | p_{ij}^{\varepsilon}(x) - p_{ij}^{\varepsilon}(y) |$. The function p_{ij}^{ε} is generally called commitor, and the hypersurfaces γ_{min,Z_i} and Γ_{min,Z_i} isocommitor surfaces.
Replacing the neighborhood of each attractor by these isocommitor surfaces, the problem is reduced to the one-dimensional case ([37]), and $\mathbb{E}(\sigma_l^{\varepsilon} - \mu_l^{\varepsilon} \mid Y_l^{\varepsilon} \notin \partial Z_j)$ is independent of l.
As above, we define $W_{ij}^{\varepsilon} = \inf\{l \mid Y_0^{\varepsilon} \in \partial\Gamma_{min,Z_i}, Y_l^{\varepsilon} \in \partial Z_j\}$ and derive the expression (7).

1.4.4 Beta approximation and isocommitor surface approximation

These isocommitor surfaces are unknown, but they can be approximated from the potential of the PDMP system within each basin, defined in the equilibrium case by the well-known

Boltzman law: $V = -\ln(\hat{u})$, $\hat{u}$ being the marginal on proteins of the stationary distribution of the process. For reasons that will be precisely the subject of next sections (studied within the context of Large deviationss), the probability $p_{ij}^{\varepsilon}(x)$ is generally linked in the weak noise limit to the function V by the relation:

$$\forall x \in Z_i : p_{ij}^{\varepsilon}(x) \underset{\varepsilon \to 0}{\sim} C_{ij} e^{V(x)/\epsilon},$$

where C_{ij} is a constant specific to each couple of basins (Z_i, Z_j).

To build such approximation, we remark that when ε is small, a cell within a basin $Z_j \in Z$ is supposed to be most of the time close to its attractor: a rough approximation could lead to identify the activation rate of a promoter e_i in each basin by the dominant rate within the basin, corresponding to the value of $k_{on,i}$ on the attractor. For any gene $i = 1, \cdots, n$ and any basin $Z_j \in Z$, we can then approximate:

$$\forall x \in Z_j : k_{on,i}(x) \approx k_{on,i}(X_{eq,Z_j}).$$

Under this assumption, the stationary distribution of the process is close to the stationary distribution of a simple two states model with constant activation function, which is a product of Beta distributions [28]. We then obtain an approximation of the marginal on proteins of the stationary distribution within each basin Z_j:

$$p_{Z_j} \approx \prod_{i=1}^{n} \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right),$$

By construction, this approximation is going to be better in a small neighborhood of the attractor X_{eq,Z_j}. Thus, this expression provides an approximation of the potential V when x is close enough from an attractor X_{eq,Z_j}:

$$V(x) \approx -\ln\left(p_{Z_j}(x)\right) \tag{8}$$

In every basin Z_i, and for all $x \in Z_i$ close to the attractor, the hypersurfaces where p_{ij}^{ε} is constant will be then well approximated by the hypersurfaces where the explicitly known function $\xi_{Z_j} = -\ln\left(\prod_{i=1}^{n} \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{dij}, \frac{k_{off,i}}{d_i}\right)\right)$ is constant.
For each attractor X_{eq,Z_i}, we can then approximate the two isocommitor surfaces described previously:

$$\begin{cases} \gamma_{min,Z_i} & \simeq \{x \in Z_i \mid \xi_{Z_i}(x) = c_1'\} \\ \Gamma_{min,Z_i} & \simeq \{x \in Z_i \mid \xi_{Z_i}(x) = c_2'\}, \end{cases} \tag{9}$$

where c_1' and c_2' are two constants such that $\xi(X_{eq,Z_i}) < c_1' < c_2'$.

1.4.5 Algorithm and limits

In the method described above, we see that the main ingredient for computing MFPTs is to compute the quantities (6). A powerful method for computing rare events numerically is given by splitting algorithms. We adapt an Adaptative Splitting Algorithm (AMS) described in [38]. Details concerning the AMS algorithm in the special case of the PDMP system can be found in Appendix D. In Section 2.5, we will verify that the probabilities given by the AMS algorithm are consistent with the ones obtained by a crude Monte-Carlo method for the toggle-switch network.
However, this quantity becomes hard to compute when both the number of genes of interest

increases and ε decreases. Indeed, the AMS algorithm allows to compute probabilities much smaller than the ones we expect for biologically relevant parameters ($\varepsilon > 0.1$), but the number of simulations needed grows at least with a polynomial factor in ε. If the number of genes considered is large, these simulations can make the algorithm impossible to run in a reasonable time. We are now going to develop an analytical approach for approximating this probability by the one of the optimal trajectory of exit from each basin, computed within the context of Large deviations.

1.5 Analytical approximation

1.5.1 Large deviations setting

In this section, we derive a variational principle for approximating the transition probability (6) introduced in Section 1.4.
A powerful methodology for rigorously deriving a variational principle for optimal paths is Large deviations theory. It has been developed extensively within the context of Stochastic Differential Equations (SDE) [39][40].
For the sake of simplicity, we present here only an heuristic version. There exists a Large deviations principle (LDP) for a stochastic process with value in Ω if for all $x_0 \in \Omega$ and a random path X_t^ε over $[0,T]$ starting at x_0, there exists a lower semi-continuous function defined on the set of continuous trajectories from $[0,T]$ to Ω, $J_T : C_{0T}(\mathbb{R}^n) \to [0,\infty]$, such that for all set of trajectories $A \subset C_{0T}(\mathbb{R}^n)$:

$$-\varepsilon \ln\left(\mathbb{P}_{x_0}^\varepsilon(X_t^\varepsilon \in A)\right) \underset{\varepsilon\to 0}{\sim} \min_{\phi\in A} J_T(\phi). \tag{10}$$

The quantity $J_T(\phi)$ is called the cost of the trajectory ϕ.

The particular application of this theory to stochastic hybrid systems has been developed in detail in [41] and [42]. We now present consequences of results developed in [43].

Definition 1. *We call Hamiltonian the function $H : \Omega \times \mathbb{R}^n \mapsto \mathbb{R}$ defined for any $(x,p) \in \Omega \times \mathbb{R}^n$, as the only eigenvalue associated to a positive right-eigenvector $\zeta(x,p)$, unique up to a normalization, of the following spectral problem:*

$$M(x,p)\zeta(x,p) = H(x,p)\zeta(x,p), \tag{11}$$

where $M(x,p) \in M_{2^n,2^n}(\mathbb{R})$ is defined by:

$$M(x,p) = \sum_{i=1}^{n} \left(K_i(x) + p_i F_i(x)\right).$$

We remark that the matrix $M(x,p)$ has off-diagonal nonnegative coefficients. Moreover, the positivity of the $k_{on,i}$ makes M irreducible (the matrix allows transition between any couple $(e,e') \in S_E^2$ after at most n steps). Thereby, the Perron Frobenius Theorem may be applied leading to existence and uniqueness of $H(x,p)$.

The following Theorem is a direct consequence of theoretical results of [43] applied to the PDMP system (1).

Theorem 1. *Let denote $\Omega^v(x) = \bigotimes_{i=1}^{n}(-d_i x_i, d_i(1-x_i))$.*
$\forall x \in \Omega$, $\forall v \in \Omega^v(x)$, the Fenchel-Legendre transform of the Hamiltonian:

$$L(x,v) = \sup_{p\in\mathbb{R}^n} \left(\langle p, v\rangle - H(x,p)\right)$$

is well defined.
Moreover, there exists a LDP for the PDMP system, and for any trajectory ϕ_t in $C_{0T}(\mathbb{R}^n)$, piecewise differentiable and verifying, for all $t \in [0,T)$, $\dot{\phi}(t) \in \Omega^v(\phi(t))$, the cost can be interpreted as a classical action, expressed as the integral of the function L called Lagrangian:

$$J_T(\phi) = \int_0^T L(\phi(t), \dot{\phi}(t))dt. \tag{12}$$

1.5.2 WKB approximation and Hamilton-Jacobi equation

The Hamiltonian defined in (11) also appears in the WKB (Wentzell, Kramer, Brillouin) approximation of the master equation [34], [43]. This approximation consists in injecting in (2) a solution of the form:

$$\forall e \in S_E,\ u_e(x,t) = \overline{\zeta}_e(x,t)e^{-\frac{\overline{V}(x,t)}{\varepsilon}}, \tag{13}$$

the underlying assumption being that the density u is non-zero on all Ω. Making a Taylor expansion in ε of the functions $\overline{V}$ and $\overline{\zeta}_e$, for any e, and keeping only the leading order terms in ε, $\overline{V}_0$ and $\overline{\zeta}_0 = (\overline{\zeta}_{0,e})_{e\in S_E}$ in the resulting master equation, we obtain:

$$-\partial_t \overline{V}_0(x,t)\overline{\zeta}_0(x,t) = \sum_{i=1}^n \left(K_i(x) + \partial_{x_i}\overline{V}_0 F_i(x)\right)\overline{\zeta}_0(x,t).$$

Identifying the vectors $\overline{\zeta}_0$ and $\nabla\overline{V}_0$ with ζ and p in (11), we obtain that $\overline{V}_0$ is solution of an Hamilton-Jacobi equation:

$$\forall x \in \Omega :\ H(x, \nabla\overline{V}_0(x,t)) + \partial_t\overline{V}_0(x,t) = 0. \tag{14}$$

We remark that (13) is reminiscent of the Gibbs distribution associated with a potential. More details about the interpretation of this equation and its link with the quasistationary approximation can be found in [34].

Suppose that a function V of class $C^1(\Omega,\mathbb{R})$ is solution of the previous equation (14). For any piecewise differentiable trajectory $\phi_t \in C_{0T}(\Omega)$ such that $\dot{\phi}(t) \in \Omega^v(\phi(t))$ for all $t \in [0,T)$, one has, by definition of the Fenchel-Legendre transform:

$$\begin{aligned}
\int_0^T L(\phi(t),\dot{\phi}(t))dt &= \int_0^T \sup_p \left(\sum_{i=1}^n p_i\dot{\phi}_i(t) - H(\phi(t),p)\right)dt \\
&\geq \int_0^T \sum_{i=1}^n \partial_{x_i}V(\phi(t),t)\dot{\phi}_i(t) - H(\phi(t),\nabla V(\phi(t),t)) \\
&= \int_0^T \sum_{i=1}^n \partial_{x_i}V(\phi(t),t)\dot{\phi}_i(t) + \partial_t V(\phi(t),t) \\
&= V(\phi(T),T) - V(\phi(0),0).
\end{aligned} \tag{15}$$

The minimum is exactly reached at any time for trajectories $\hat{\phi}_t$ solutions for all $t \in [0,T)$ of the system defined for all $i = 1,\cdots,n$ by:

$$\begin{cases} p_i(t) &= \partial_{x_i}V(\hat{\phi}(t),t) \\ \dot{\hat{\phi}}_i(t) &= \partial_{p_i}H(\hat{\phi}(t),p(t)). \end{cases} \tag{16}$$

Thus, a regular solution V of the Hamilton-Jacobi equation (14) defines trajectories minimizing the cost between any couple of its points. If trajectories solutions of the system (16) realize events described by the probabilities (6), these probabilities can be approximated by the costs of these trajectories, as they realize the minimum in the expression (10).

2 Results

2.1 Expressions of the Hamiltonian and the Lagrangian

In this section, we identify the Perron eigenvalue $H(x,p)$ of the spectral problem (11), and prove that its Fenchel-Legendre transform L, with respect to the variable p is well defined on $\mathbb{R}^n$. We then obtain the explicit form of the Hamiltonian and the Lagrangian associated to the PDMP system.

Theorem 2. *For all n in $\mathbb{N}^*$, the spectral problem (11) has a unique eigenvalue associated to a positive eigenvector, defined for every (x,p) by the function:*

$$H(x,p) = \frac{1}{2}\sum_{i=1}^{n}\Big(p_i d_i(1-2x_i) - (k_{on,i}(x)+k_{off,i}) + \sqrt{(p_i d_i + k_{on,i}(x) - k_{off,i})^2 + 4k_{on,i}(x)k_{off,i}}\Big). \tag{17}$$

Moreover, the function H is convex with respect to p.

Theorem 3. *Let denote $\overline{\Omega^v}(x) = \bigotimes_{i=1}^{n}[-d_i x_i, d_i(1-x_i)]$.*
The Lagrangian of the PDMP system is defined for every $(x,v) \in \Omega \times \mathbb{R}^n$ by the function:

$$\begin{cases} L(x,v) = \sum_{i=1}^{n}\left(\sqrt{k_{off,i}\frac{v_i + d_i x_i}{d_i}} - \sqrt{k_{on,i}(x)\frac{d_i(1-x_i)-v_i}{d_i}}\right)^2 & \text{if } v \in \overline{\Omega^v}(x) \\ L(x,v) = \infty & \text{if } v \notin \overline{\Omega^v}(x) \end{cases} \tag{18}$$

In addition, for all $x \in \Omega$, $L(x,v) = 0$ if and only if for all $i = 1, \cdots, n$:

$$v_i = d_i\left(\frac{k_{on,i}(x)}{k_{on,i}(x)+k_{off,i}} - x_i\right).$$

Proof of Theorem 2. We begin by the analysis of the case of one gene to understand the method. In this case, the desired eigenvector is only 2-dimensional ($\mathrm{card}(S_E) = 2$), and can be rewritten for all $x \in \Omega$ as $\zeta_1(x) = \alpha_p(x)$ and $\zeta_0(x) = 1 - \alpha_p(x)$ with $\alpha_p(x) \in (0,1)$. The problem can be rewritten as:

$$\begin{cases} -k_{on}(x)(1-\alpha_p(x)) + k_{off}\alpha_p(x) &= (H(x,p) + pdx)(1-\alpha_p(x)) \\ k_{on}(x)(1-\alpha_p(x)) - k_{off}\alpha_p(x) &= (H(x,p) - pd(1-x))\alpha_p(x). \end{cases}$$

First we remark that it is necessary to have $\forall x,p \in \Omega \times \mathbb{R}^n$, $\alpha_p(x) \in (0,1)$ because k_{on} and k_{off} are positive.
By summing the two equations, we obtain:

$$H(x,p) = pd(\alpha_p(x) - x). \tag{19}$$

We can then reduce the problem to one equation:

$$T(\alpha) = k_{off}\alpha_p(x) - k_{on}(x)(1-\alpha_p(x)) + dp(\alpha_p(x)-1)\alpha_p(x) = 0.$$

This equation has two solutions in $\mathbb{R}$. As $T(0) = -k_{on}(x)$ and $T(1) = k_{off}$, T has one and only one root in $(0,1)$, for all $x,p \in \Omega \times \mathbb{R}$, which can be written:

$$\begin{cases} \alpha_p(x) = \frac{1}{2}\left(1 + \frac{\sqrt{(pd + k_{on}(x) - k_{off})^2 + 4k_{on}(x)k_{off}} - (k_{on}(x) + k_{off})}{pd}\right) & \text{if } p \neq 0 \\ \alpha_p(x) = \frac{k_{on}(x)}{k_{on}(x) + k_{off}} & \text{if } p = 0 \end{cases}$$

We then obtain:
$H(x,p) = \frac{1}{2}\left(pd(1-2x) - (k_{on}(x) + k_{off}) + \sqrt{(pd + k_{on}(x) - k_{off})^2 + 4k_{on}(x)k_{off}}\right)$.

The case of n genes is a generalization of this reasoning, which uses the tensorial expression of the matrices $K_i(x)$ and $F_i(x)$.

We define a vector $\alpha_p(x) = (\alpha_{p,1}(x), \cdots, \alpha_{p,n}(x)) \in \mathbb{R}^n$ where for all $i = 1, \cdots, n$, $\alpha_{p,i}$ is defined as in the one-dimensional case:

$$\begin{cases} \alpha_{p,i}(x) = \frac{1}{2}\left(1 + \frac{\sqrt{(p_i d_i + k_{on,i}(x) - k_{off,i})^2 + 4k_{on,i}(x)k_{off,i}} - (k_{on,i}(x) + k_{off,i})}{p_i d_i}\right) & \text{if } p_i \neq 0 \\ \alpha_{p,i}(x) = \frac{k_{on,i}(x)}{k_{on,i}(x) + k_{off,i}} & \text{if } p_i = 0 \end{cases} \tag{20}$$

We now define the vector:

$$\psi(x,p) = \bigotimes_{i=1}^{n} \begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix}.$$

The vector ψ is always non-negative, since for all $x \in \Omega$, $\alpha_{p,i}(x) \in (0,1)$ for all $i = 1, \ldots, n$.
Setting

$$H_i(x,p_i) = \frac{1}{2}(p_i d_i(1-2x_i) - (k_{on,i}(x) + k_{off,i}) + \sqrt{(p_i d_i + k_{on,i}(x) - k_{off,i})^2 + 4k_{on,i}(x)k_{off,i}}),$$

we clearly have for all i, from the one-dimensional case:

$$\left(K^{(i)}(x) + p_i F^{(i)}(x)\right)\begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix} = H_i(x,p_i)\begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix}$$

We can take a tensorial product and sum on i, to obtain:

$$\sum_{i=1}^{n} \begin{pmatrix} 1 - \alpha_{p,1}(x) \\ \alpha_{p,1}(x) \end{pmatrix} \otimes \cdots \otimes \underbrace{\left(K^{(i)}(x) + p_i F^{(i)}(x)\right)\begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix}}_{\text{i-th position}} \otimes \cdots$$
$$\otimes \begin{pmatrix} 1 - \alpha_{p,n}(x) \\ \alpha_{p,n}(x) \end{pmatrix} = \left(\sum_{i=1}^{n} H_i(x,p_i)\right) \bigotimes_{i=1}^{n} \begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix}$$

which is exactly the tensorial expression of the spectral problem (11) for the vector $\zeta = \psi$ defined above.
We retrieve the form (17) for the Hamiltonian: $H(x,p) = \sum_{i=1}^{n} H_i(x,p_i)$.

To see the convexity of H with respect to p, we verify that, for all $i = 1, \cdots, n$:

$$\frac{\partial^2}{\partial p_i^2} H(x,p) = \frac{2d_i^2 k_{on,i}(x)k_{off,i}}{(\sqrt{(p_i d_i + k_{on,i}(x) - k_{off,i})^2 + 4k_{on,i}(x)k_{off,i}})^{\frac{3}{2}}} > 0.$$

□

Proof of Theorem 3. We now identify the Fenchel-Legendre transformation of the Hamiltonian defined in Theorem 2.

For all $x \in \Omega$ and for all $v_i \in \mathbb{R}$, the function $g : p_i \mapsto p_i v_i - H_i(x, p_i)$ is concave. An asymptotic expansion gives:

$$g(p_i) = p_i \left(v_i - d_i \left(\frac{1}{2} \left(1 + \mathrm{sgn}(p_i) \right) - x_i \right) \right) + \frac{1}{2} \left(k_{on,i}(x) + k_{off,i} + \mathrm{sgn}(p_i) \left(k_{on,i}(x) - k_{off,i} \right) \right) + O\left(\frac{1}{p_i} \right).$$

If $v_i \in (-d_i x_i, d_i(1 - x_i))$, g goes to $-\infty$ when $p_i \to \pm\infty$: thus g reaches an unique maximum in $\mathbb{R}$. At the limit $v_i = -d_i x_i$ or $d_i(1 - x_i)$, g goes to $-\infty$ at one side and is bounded on the other side: then g is upper bounded and the sup is well defined. If $v_i \notin [-d_i x_i, d_i(1 - x_i)]$, g is clearly not bounded.
As a consequence, $L_i(x, v_i) = \sup_{p_i} \left(p_i v_i - H_i(x, p_i) \right)$ is well defined and finite in the domain $\Omega \times [-d_i x_i, d_i(1 - x_i)]$.
The Fenchel-Legendre transformation is then well defined for all $x \in \Omega$ and $v \in \overline{\Omega^v}(x)$ by:

$$L(x, v) = \sum_i L_i(x, v_i) = \sum_{i=1}^{n} \sup_{p_i \in \mathbb{R}} \left(p_i v_i - H_i(x, p_i) \right),$$

and the sup is reached inside $\mathbb{R}^n$ for every $v \in \Omega^v(x)$. To find an expression for $L(x, v)$, we have to find for all $i = 1, \cdots, n$ the unique solution $\overline{p_i} = \overline{p_{v,i}}(x)$ of the invertible equation: $v_i = \frac{\partial H_i}{\partial p_i}$.

$$v_i = \frac{\partial H_i}{\partial p_i}(x, p_i) = \frac{1}{2} \left(d_i(1 - 2x_i) + \frac{d_i(d_i p_i + k_{on,i}(x) - k_{off,i})}{\sqrt{(d_i p_i + k_{on,i}(x) - k_{off,i})^2 + 4 k_{on,i}(x) k_{off,i}}} \right)$$
$$\iff u_i = \frac{d_i z_i}{\sqrt{z_i^2 + c_i}},$$

where $u_i = 2(v_i + d_i x_i) - d_i$, $c_i = 4 k_{on,i}(x) k_{off,i} > 0$, $z_i = d_i p_i + k_{on,i}(x) - k_{off,i}$.
When $v_i \in (-d_i x_i, d_i(1 - x_i))$, we have $u_i \in (-d_i, d_i)$. Thus, we obtain:

$$z_i = \pm \frac{u_i \sqrt{c_i}}{\sqrt{d_i^2 - u_i^2}}.$$

As z_i and u_i must have the same sign, we can conclude for every $v_i \in (-d_i x_i, d_i(1 - x_i))$:

$$\overline{p}_i = \frac{1}{d_i} \left(k_{off,i} - k_{on,i}(x) + \sqrt{k_{off,i} k_{on,i}(x)} \frac{2(v_i + d_i x_i) - d_i}{\sqrt{(v_i + d_i x_i)(d_i(1 - x_i) - v_i)}} \right). \tag{21}$$

Injecting this value of $p_i = \overline{p}_i$ in the Fenchel-Legendre transform, we obtain:

$$\begin{aligned} L_i(x, v_i) &= \overline{p}_i v_i - H_i(x_i, \overline{p}_i) \\ &= \frac{1}{2} \left(\sqrt{2 k_{off,i} \frac{v_i + d_i x_i}{d_i}} - \sqrt{2 k_{on,i}(x) \frac{d_i(1 - x_i) - v_i}{d_i}} \right)^2. \end{aligned}$$

We finally obtain the expression for any $v \in \Omega^v(x)$:

$$L(x, v) = \sum_{i=1}^{n} \left(\sqrt{k_{off,i} \frac{v_i + d_i x_i}{d_i}} - \sqrt{k_{on,i}(x) \frac{d_i(1 - x_i) - v_i}{d_i}} \right)^2.$$

Passing to the limit, we extend this expression for $v \in \overline{\Omega^v}(x)$. As expected, the Lagrangian is always non-negative. From the asymptotic expansion presented above, we complete this expression by the value $+\infty$ outside $\overline{\Omega^v}(x)$.
In addition, we immediately see that $L(x,v) = 0$ if and only if the velocity field v characterizes the deterministic trajectories, solutions of the system (4). □

2.2 Stationary Hamilton-Jacobi equation

We justified in Section 1.5.2 that solutions of the Hamilton-Jacobi equation (14) are central for finding an analytical approximation of the MFPTs described in Section 1.4. Non-stationary solutions being generally out of range, we are going to restrict our study to the stationary Hamilton-Jacobi equation, i.e to study functions $V \in C^1(\Omega, \mathbb{R})$ such that for all $x \in \Omega$:

$$H(x, \nabla V(x)) = 0. \tag{22}$$

Recall that, $H(x,p) = \sum_{i=1}^{n} p_i d_i(\alpha_{p,i}(x) - x_i)$, two classes of solutions are obviously given by functions V such that for all $i = 1, \cdots, n$, $p_i = \partial_{x_i} V$, and for all $x \in \Omega$: $p_i(x) = 0$ or $\alpha_{p,i}(x) = x_i$.
The first class contains every constant function on Ω. From the expression (20), they are associated to a probability of promoters states defined for all $i = 1, \cdots, n$ and for all $x \in \Omega$ by: $\alpha_i(x) = \frac{k_{on,i}(x)}{k_{off,i} + k_{on,i}(x)}$.
From the expression (20), the second class contains all functions V such that for all $x \in \Omega$:

$$\forall i = 1, \cdots, n : \partial_{x_i} V(x) = -\frac{k_{on,i}(x)}{d_i x_i} + \frac{k_{off,i}}{d_i(1 - x_i)}, \tag{23}$$

We show in Appendix B that this relation is hold at least for a simple class of networks including the toggle-switch described in Appendix C.
We will see in the next section that the class of constant solutions defines the deterministic system (4). A generalization of the second class of solutions (23) defines the optimal trajectories of exit from the basins of attraction of the deterministic system, and is consistent with the interpretation of the distribution $e^{-\frac{V(\cdot)}{\varepsilon}}$ as an approximation of the marginal on proteins of the steady state distribution.

2.3 Optimal trajectories

We denote $C_{0T}^{1,pw}(\Omega)$ the set of piecewise differentiable trajectories in $C_{0T}(\Omega)$.

Definition 2. *We define the quasipotential as follows: for two sets $A, B \subset \Omega$ and a set $R \subset \Omega \setminus (A \cup B)$,*

$$Q_R(A,B) = \inf_{\phi_t, T} \{J_T(\phi) \mid \phi_t \in C_{0T}^{1,pw}(\Omega), \phi(0) \in A, \phi(T) \in B, \forall t \in (0,T) : \phi(t) \notin R\}.$$

We call optimal trajectory between the two subsets $A, B \subset \Omega$, in $\Omega \setminus R$, a trajectory $\phi_t \in C_{0T}^{1,pw}(\Omega)$ for which the previous infimum is reached.

For the sake of simplicity, if $R = \varnothing$, we write $Q_R(A,B) = Q(A,B)$.

The following lemma is a direct consequence of the inequality (15), in the case where equality is reached:

Lemma 1. *For a solution $V \in C^1(\Omega, \mathbb{R})$ of (22), any trajectory $\phi_t \in C_{0T}^{1,pw}(\Omega)$ solution of the system (16) is optimal in Ω between each couple of points $(\phi(t_1), \phi(t_2))$ such that $T \geq t_2 > t_1 \geq 0$, and we have:*

$$Q(\phi(t_1), \phi(t_2)) = V(\phi(t_2)) - V(\phi(t_1)).$$

We are now going to study some properties of the optimal trajectories associated to two classes of solutions of the equation (22).

2.3.1 Deterministic limit and forward trajectories

From Lemma 1, every constant solution of (22) is associated to a collection of paths ϕ_t, optimal in Ω. These trajectories are solutions of the deterministic limit system (4) defined for every $t > 0$ by:

$$\forall i \in \{1, \cdots, n\} : \dot{\phi}_i(t) = d_i \left(\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t)) + k_{off,i}} - \phi_i(t) \right).$$

Moreover, for every trajectory ϕ_t solution of this system, we have for any $T > 0$:

$$J_T(\phi_t) = \int_0^T L(\phi_t, \dot{\phi}_t) dt = 0. \tag{24}$$

We call such trajectories the *forward trajectories*, as they characterize the optimal path of convergence within every basins. From Theorem 3, these forward trajectories are the only zero-cost admissible trajectories.

2.3.2 Quasipotential and backward trajectories

We now characterize the optimal trajectories of exit from the basins. For this sake, we are going to find a sufficient condition for a solution of the equation (22) of class $C^1(\Omega, \mathbb{R})$ to define the optimal trajectories associated to the probabilities (6).

Definition 3. *We define the following condition on a function $V \in C^1(\Omega, \mathbb{R})$:*

(C) The gradient of V vanishes only on isolated points of Ω.

In the first theorem (Theorem 4), we state some properties of solutions V of (22) of class $C^1(\Omega, \mathbb{R})$ satisfying condition (C):

Theorem 4. *Let V a solution of (22) of class $C^1(\Omega, \mathbb{R})$.*

(i) For any optimal trajectory ϕ_t of the system (16), we have the equivalence for any time t:

$$\left(\forall i \in \{1, \cdots, n\}, \, \phi_i(t) = \frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t)) + k_{off,i}} \right) \iff \dot{\phi}(t) = 0.$$

(ii) The condition (C) is equivalent to the condition:

(C') the gradient of V vanishes only on the stationary points of the system (4).

(iii) If V satisfies (C), V strictly increases on every trajectory solution of the system (16) which does not begin at an equilibrium point of the system (4).
Moreover, for any basin of attraction Z_i of the system (4) associated to an attractor X_{eq,Z_i}, we have:

$$\forall x \in Z_i \setminus X_{eq,Z_i}, \, V(x) > V(X_{eq,Z_i}).$$

(iv) For any solution $\tilde{V}$ solution of (22) in $C^1(\Omega, \mathbb{R})$ and satisfying the condition (C), there exists a constant $c \in \mathbb{R}$ such that: $\forall x \in \Omega$, $V_1(x) - V_2(x) = c$.

We remark that any function verifying the relation (23) belongs to the class of solutions of (22) satisfying the condition (C).
The precise analysis of the existence of a regular solution V verifying (C) for a given network is beyond the scope of this article. We remark that the points (iii) and (iv) make these solutions consistent with the interpretation of such function V coming from the WKB approximation, for which the distribution $u(\cdot) = e^{-\frac{\overline{V}(\cdot)}{\varepsilon}}$ approximates the stationary distribution of the PDMP system. The condition (C) appears as a natural extension of the condition stated in Section 1.2 for which the equilibrium of the deterministic system are reduced to single points.

If $V \in C^1(\Omega, \mathbb{R})$ is solution of the equation (22) and satisfies (C), we call a trajectory $\phi_t \in C^{1,pw}_{0T}(\Omega)$ solution of the system (16) a *backward trajectory*.
When $p = \nabla V$ verifies the relation (23), we can explicitly describe these trajectories, injecting this expression in the relation $v = -\frac{\partial H}{\partial p}(x, p)$. For all $t \in [0, T)$:

$$\forall i \in \{1, \cdots, n\} : \dot{\phi}_i(t) = d_i \left(\frac{k_{off,i}\phi_i(t)^2}{k_{on,i}(\phi(t))(1-\phi_i(t))^2 + k_{off,i}\phi_i(t)^2} - x_i \right). \tag{25}$$

In the second theorem (Theorem 5), we justify that the backward trajectories are the optimal trajectories of exit from each basin.

Theorem 5. *Let assume that there exists $V \in C^1(\Omega, \mathbb{R})$ solution of (22) and satisfying the condition (C).*
Let consider a couple of basins (Z_i, Z_j) of the system (4) such that $\partial Z_i \cap \partial Z_j \neq \varnothing$. We denote X_{eq,Z_i} the attractor of Z_i.
For all y in $\partial Z_i \cap \partial Z_j$ and for all $r > 0$, there exists a couple (x, ϕ), where $||x - X_{eq,Z_i}||^2 < r$ and ϕ is a backward trajectory, such that $\phi(0) = x$, $\phi(T) \underset{T\to\infty}{\to} y$.
Denoting $R = \bigcup_{k\neq j} \{\partial Z_i \cap \partial Z_k\}$, we have:

$$Q_R(X_{eq,Z_i}, \partial Z_i \cap \partial Z_j) = \min_{y \in \partial Z_i \cap \partial Z_j} V(y) - V(X_{eq,Z_i}).$$

We denote $X_{un,ZiZj} = \arg\min\{V(y) \mid y \in \partial Z_i \cap \partial Z_j, \nabla V(y) = 0\}$. If any forward trajectory starting in $\partial Z_i \cap \partial Z_j$ stays in $\partial Z_i \cap \partial Z_j$ and converges to one of the saddle points, then:

$$\forall y \in X_{un,ZiZj}, : Q_R(X_{eq,Z_i}, \partial Z_i \cap \partial Z_j) = V(y) - V(X_{eq,Z_i}).$$

The proofs of these two theorems use classical tools from Hamiltonian system theory and can be found in Appendix E.

When a solution V satisfying (C) exists, the saddle points of the deterministic system (4) are then generally the bottleneck of transitions between basins and the function V characterizes the energetic barrier between them.
The function $Q(X_{eq,Z_i}, \cdot)$ depends on the basin Z_i, which is a local property: it explains why the function V is generally called the global quasipotential, and $Q(X_{eq,Z_i}, \cdot)$ the local quasipotential of the stochastic process [44].

2.4 Partial conclusion

We have obtained in Theorem 3 a variational expression for the cost function J_T of the Large deviations principle for the PDMP system (1). We have also highlighted the existence and meaning of two types of optimal trajectories.
Let $x \in Z_i$ and $y \in \Omega$ such that $Q(x, y) > 0$. If we denote A the set of trajectories beginning at x and reaching a small neighborhood of X_{eq,Z_i} before y, we see that a forward trajectory

beginning at x satisfies obviously this event in a finite time. From the expression (10), the cost of the forward trajectories being equal to 0, we have then:

$$\mathbb{P}_x^\varepsilon(X_t^\varepsilon \in A) \underset{\varepsilon\to 0}{\sim} 1.$$

This justifies that, for all basin $Z_i \in Z$ and $\forall x \in Z_i$, a random path starting at x reaches X_{eq,Z_i} before every point $y \in \Omega$ such that $Q(x,y) > 0$, with probability 1 when $\varepsilon \to 0$.
For any basin Z_j, $i \neq j$, the probability (6), which is the probability of reaching Z_j from Γ_{min,Z_i}, before γ_{min,Z_i}, $p_{ij}^\varepsilon(x)$, can be approximated by the expression:

$$-\varepsilon \ln\left(p_{ij}^\varepsilon(x)\right) \underset{\varepsilon\to 0}{\sim} Q_{\bigcup\limits_{k\neq j}\{\partial Z_i \cap \partial Z_k\}}(X_{eq,Z_i}, \partial Z_i \cap \partial Z_j).$$

When there exists a function V belonging to the second class of obvious solutions of the equation (22), i.e verifying (23), we can conclude explicitly, injecting the expression of v given by (25) in the expression (18):

$$\varepsilon \ln\left(\mathbb{E}(W_{ij}^\varepsilon)\right) \underset{\varepsilon\to 0}{\sim} \lambda_{i,j} = \int_0^T \sum_{i=1}^n \frac{\left(k_{on,i}(\phi(t))(1-\phi_i(t)) - k_{off,i}\phi_i(t)\right)^2}{k_{on,i}(\phi(t))(1-\phi_i(t))^2 + k_{off,i}\phi_i(t)^2} dt, \tag{26}$$

where W_{ij}^ε is defined in Section 1.4, and $\phi(t) \in C^{1,pw}(\Omega)$ is solution of the system (25) for an appropriate initial condition close from X_{eq,Z_i}, reaching the minimum of V on the common boundary $\partial Z_i \cap \partial Z_j$ at $T > 0$. As proved in Theorem 5, if any forward trajectory starting in $\partial Z_i \cap \partial Z_j$ stays inside, this minimum is reached on a saddle point of V on $\partial Z_i \cap \partial Z_j$ and $T \sim \infty$. The continuity of V ensures that the initial condition, if close enough (and not equal) to X_{eq,Z_i}, has no impact on the estimated value of $\ln(\mathbb{E}(W_{ij}^\varepsilon))$: we need to choose the isocommitor surface Γ_{min,Z_i}, from which we compute the probability of exit, such that $\sup\limits_{x\in\Gamma_{min,Z_i}} \{(X_{eq,Z_i} - x)^2\}$ is sufficiently small (see Section 1.4, and Appendix D).
Thus, we can conclude:

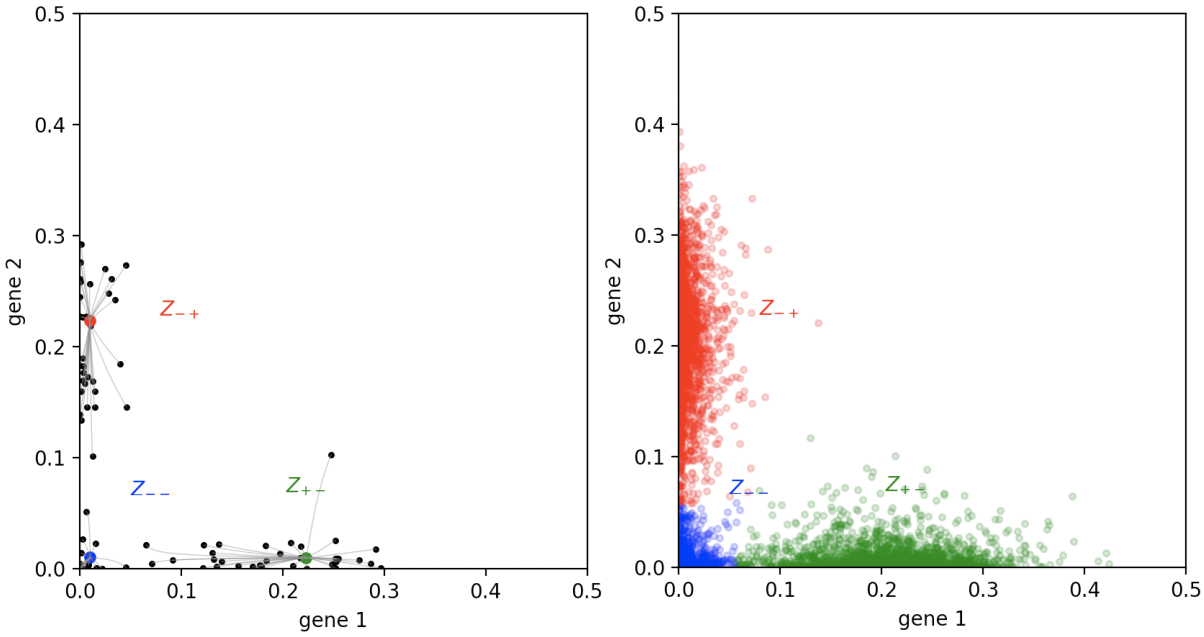
$$\overline{T}_{Z_i,Z_j}^\varepsilon \simeq C_{ij} e^{\frac{\lambda_{ij}}{\varepsilon}} \overline{T}_{Z_i,Z_i}^\varepsilon, \tag{27}$$

where C_{ij} is an appropriate prefactor which is supposed to not depend on ε. Unfortunately, if there exists an explicit expression of C_{ij} in the one-dimensional case ([34]), and that an approximation has been built for multi-dimensional SDE model [45], they seem intractable in our case. A solution consists in approximating the prefactor by comparison between the probabilities given by the AMS algorithm and the Large deviations approximation (27) for a fixed ε, using then the linearity of $-\ln\left(\overline{T}_{Z_i,Z_j}^\varepsilon\right)$ with respect to ε.

To conclude, for every couple of basins $(Z_i, Z_j), i \neq j$, one of the most efficient methods for computing the probability (6) is to use the AMS algorithm. When the number of dimensions is large, coupled with a ε too small for this algorithm to be efficiently run, we have to use the LDP approximation (27). The AMS algorithm is therefore necessary for approximating the prefactors, but can be run for a larger ε. Finally, the quantity $\overline{T}_{Z_i,Z_i}^\varepsilon$ can be computed by a crude Monte-Carlo method to conclude on the value of the MFPT $\overline{T}_{Z_i,Z_j}^\varepsilon$.

2.5 Application to the toggle-switch network

In this part, we consider the class of interaction functions defined in Appendix B for a network of two genes ($n = 2$). This function comes from a chromatin model developed in [28] and is consistent with the classical Hill function characterizing promoters switches. Using results and methods described in the previous sections, we are going to reduce the PDMP system



(a) (b)

Figure 6: 6a: 100 cells are plotted under the stationary distribution. The forward trajectory bound each of these cells to its associated attractor. 6b: 5000 cells are classified depending on their attractor with this procedure. This figure sketches the different basins of attraction, the ratio of cells between each of them and the distribution of proteins concentration inside.

for parameters characterizing the toggle-switch network described in Appendix C. We compare the results, i.e the parameters characterizing the discrete Markov chain on the cellular types approximated with Large deviations theory and the method described in Section 1.4, to MFPTs given by a crude Monte-Carlo method.

2.5.1 Computation of the attractors, saddle points and optimal trajectories

First, we compute the stable equilibrium points of the PDMP system (1). The problem (5) has no explicit solution: we present a simple method to compute them.
It consists in sampling a collection of random paths in Ω: the distribution of their final position after a long time approximates the marginal on proteins of the stationary distribution. We use these final positions as starting points for simulating the forward trajectories, described by (4), with an ODE solver: each of these forward trajectories converges to one of the stable equilibrium points. This method allows to obtain all the stable equilibrium corresponding to sufficiently deep potential wells (see Figure 6). Possible other potential wells can be omitted because they correspond to basins where the process has very low probability of going, and which does not impact significantly the Markov chain of the coarse-grained model.
Second, we need to find the saddle points. In general, the reverse of the forward trajectories, as the backward trajectories (given by (25) if the condition (23) holds), are very unstable: the saddle point cannot be obtained with a direct shooting method around the stable equilibrium. Many methods are available for computing saddle points when the energetic landscape is known [46]. We develop in our case a simple algorithm which uses the Lagrangian associated to the trajectories defined by (25). This Lagrangian, defined on the right-hand side of (26), is a non-negative function which vanishes only at the stationary points of the deterministic limit (4). If there exists a saddle point connecting two attractors, this function will then vanish at this point. The algorithm is described in Appendix F. For the toggle-switch network, this method allows to recover all the saddle points.

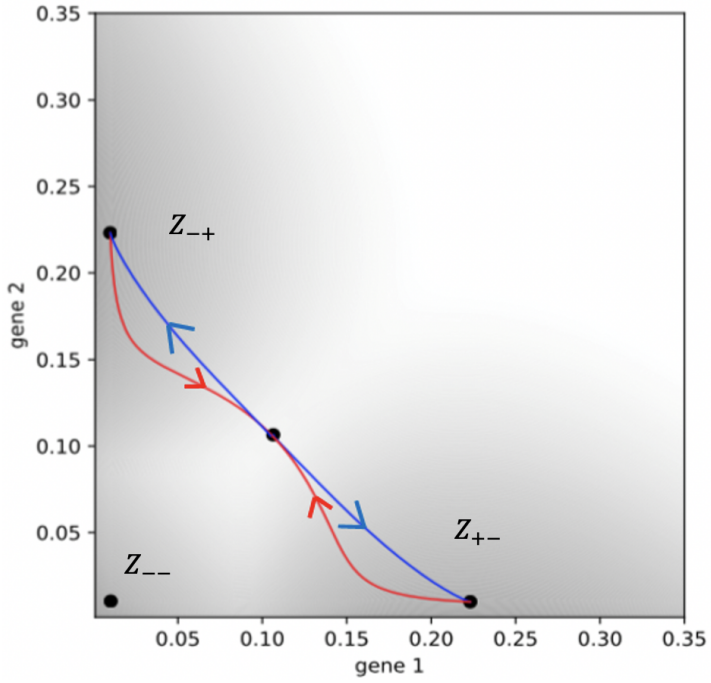
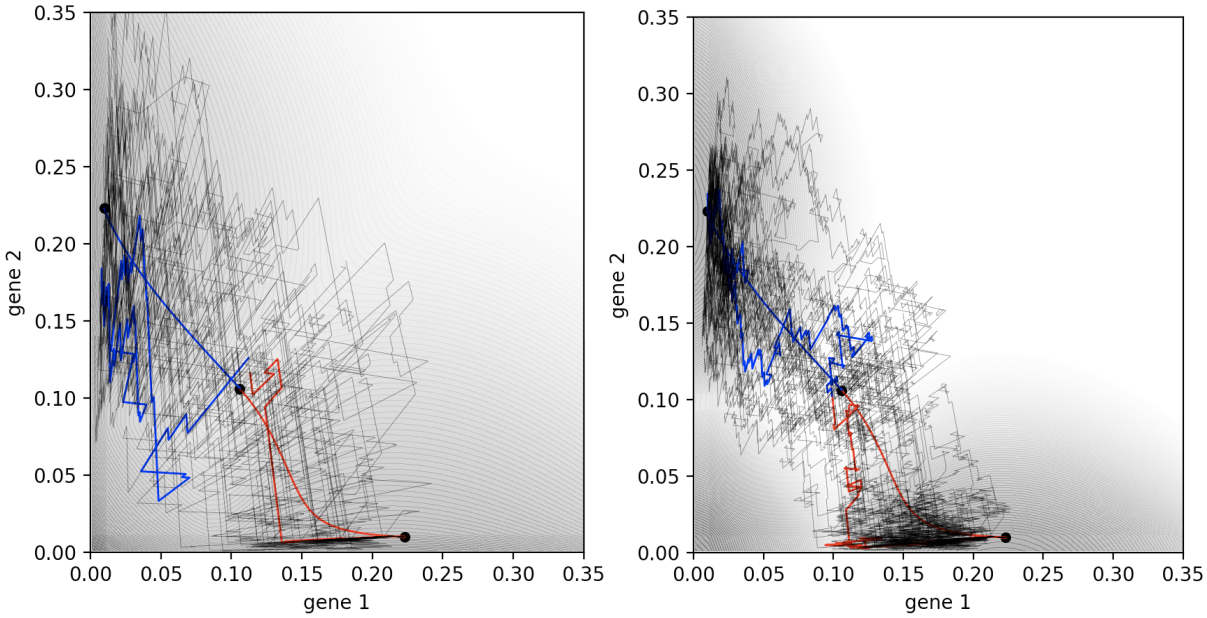


Figure 7: The optimal backward trajectories from a first attractor continued by the forward trajectories reaching the second attractor, for the pair (Z_{+-}, Z_{-+}). We omit the other pairs of attractors (Z_{+-}, Z_{--}) and (Z_{--}, Z_{-+}), because their optimal trajectories are simply straight lines.

We prove in Appendix B that for any two-dimensional network associated to such interaction function, if the network is symmetric, i.e that for any couple of genes (i, j), $\theta_{ij} = \theta_{ji}$ (where θ is the matrix characterizing the interactions between genes), there exists a function V such that the relation (23) is verified.
The toggle-switch network being symmetric, such function V exists and is then solution of the Hamilton-Jacobi equation. Moreover, we observe that any forward trajectory starting in the common boundary of two basins stays on this boundary: from Theorem 5, the trajectories solutions of the system (25) between the attractors and the saddle points characterize the optimal cost of exit from every basin.
Using the reverse of the backward trajectories, for which the attractors are necessarily attractive (see the proof of Theorem 4.(iv)), we can apply successively a shooting method around every saddle point to plot the backward trajectories.

For any pair of basins (Z_i, Z_j), we then obtain the optimal trajectories connecting the stable equilibrium points X_{eq,Z_i} and the saddle points belonging to the common boundary $\partial Z_i \cap \partial Z_j$ (see Figure 7). Their costs are explicitly described by the right-hand side of the expression (26). From Theorem 5, the optimal trajectories of exit are necessarily the ones minimizing the cost among all these trajectories. We observe that for the toggle-switch network, there is only one optimal trajectory between each pair of basins (Z_i, Z_j), that we denote $\phi_t^{Z_i Z_j}$.

The Large deviations setting ensures that $\forall \delta, \eta > 0$, $\exists \bar{\varepsilon} > 0$, such that $\forall \varepsilon < \bar{\varepsilon}$, a random path X_t^ε reaching Z_j from Γ_{Z_i} before γ_{Z_i}, verifies: $\sup_t\{|\ X_t^\varepsilon - \phi_t^{ij}\ |\} \leq \delta$ with probability greater than $1 - \eta$. We could then theoretically find ε such that any trajectory of exit from Z_i to Z_j would be indistinguishable from trajectory $\phi_t^{Z_i Z_j}$. But in practice, it is almost impossible to



(a) **(b)**

Figure 8: Comparison between the optimal trajectory of the figure 7 and 30 random paths conditioned on reaching $X_{eq,Z_{+-}}$ before going back to γ_{-+} from a random point of $\partial\Gamma_{-+}$, for 8a : $\varepsilon = 1/7$ and 8b : $\varepsilon = 1/21$. For each figure, one of these random paths is colored, separating the backward and the forward parts.

observe such random paths just by simulation: for ε small enough, the event $\{\tau^{\varepsilon}_{Z_j} < \tau^{\varepsilon}_{\gamma_{Z_i}}\}$ is too rare to be simulated.
We plot in Figure 8 two sets of random exit paths, simulated for two different ε, illustrating the fact that the probability of an exit path to be far from the optimal backward trajectory decreases with ε.

2.5.2 Comparison between predictions and simulations

For each couple of basins (Z_i, Z_j), the expression (26) provides an approximation of the probability of the rare event $\{\tau^{\varepsilon}_{Z_j} < \tau^{\varepsilon}_{\gamma_{Z_i}}\}$, up to a prefactor, and expression (27) allows to deduce the associated MFPT.
We plot in Figure 9 the evolution of these two estimations, as ε decreases, comparing respectively to the probabilities given by the AMS algorithm (on the left) and the MFPTs computed with a standard MC method (to the right). As in [47], we decide to plot these quantities in logarithmic scale. We observe that, knowing the prefactor, the Large deviations approximation is accurate even for $\varepsilon > 0.1$, and that induced MFPTs are close to the ones observed with a crude Monte-Carlo method. We represent in Figure 11b the variance of the estimator of the MFPTs given by the AMS method.

We also remark that our analysis provides two ways of finding the stationary measure of the discrete coarse-grained model: on the one hand, computing the stationary distribution μ_z of the Markov process $(\overline{Z}_l)$, on the other hand, taking the ratio μ_b, from many cells under the long-time proteins distribution obtained from simulations of the PDMP system (1), of the ones belonging to each basins (see Figure 10).
The computational time needed for approximating the MFPTs with a MC method being very high for small ε, comparing these two stationary distributions appears as a good alternative for verifying the accuracy of the transition rates approximations. We plot in Figure 11a the

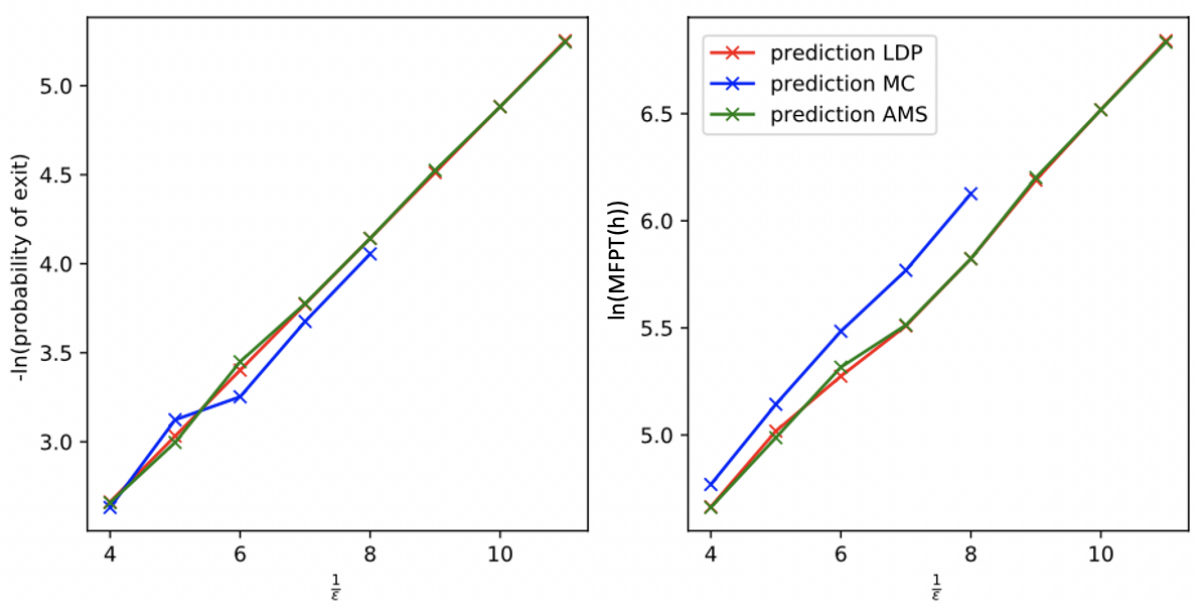


Figure 9: Comparison between probabilities given by the Large Deviation expression and the AMS algorithm, in logarithmic scale, between the basins Z_{+-} and Z_{--}. We compute the prefactor and adjust the curves to fit the numerical results. The Monte-Carlo method is not represented after $\varepsilon = \frac{1}{8}$ because the MFPT becomes too large to be efficiently computed.

evolution of the total variation of the difference between these two stationary distributions as ε^{-1} increases. The total variation is small even for realistic values of ε.
The variance of the estimator of μ_b is very small (given it is estimated after a time long enough) but the estimator of μ_z accumulates all numerical errors coming from the estimators needed to compute each MFPT, which explain the unexpected small increases observed in the curve for $\varepsilon = 1/6$. We represent in Figure 11b the variance of the MFPTs estimators between pair of attractors used for estimating the distribution μ_z in Figure 11a, for $\varepsilon = 1/7$: as expected, this variance increases with the MFPTs.

The similarity between the two distributions μ_z and μ_b seems to justify the Markovian approximation of the reduced process $\hat{Z}_t^\varepsilon$ for small but realistic ε: at least for the toggle-switch network, the coarse-grained model, evolving on the basins of attractions seen as cellular types, describes accurately the complex behaviour of a cell in the gene expression space.

2.6 Mixture model

Combining the approximation of the functions $k_{on,i}$ by their main mode within a basin, described in Section 1.4.4, with the description of metastability provided in Section 1.3, we now consider another process described by the $2n+1$-dimensional vector of variables $(Z(t), E(t), X(t))$, representing respectively the cell type, the promoter state and the protein concentration of every gene (see Figure 12).
Considering that the PDMP process spends in each basin a time long enough to equilibrate inside, we decide to approximate the distribution of a couple $(E(t), X(t))$ in a basin Z_j by its quasistationary distribution. As referred in Section 1.4.4, it is then equivalent to the stationary distribution of a simple two states model with constant activation function, which is a product of Beta distributions. We can then approximate the marginal on proteins of the stationary

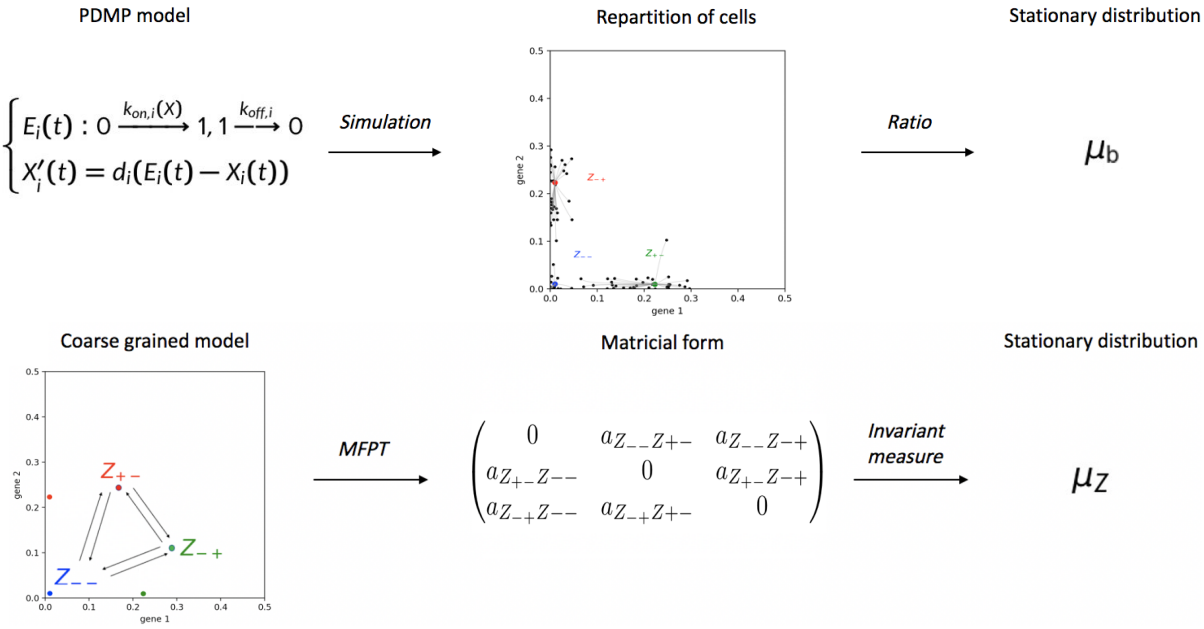
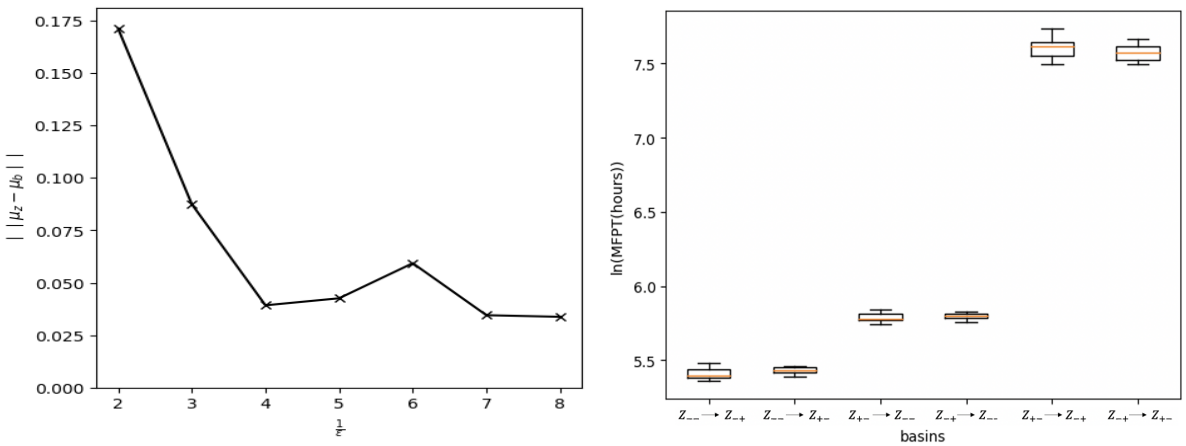


Figure 10: Comparison between the two methods for obtaining the stationary distributions on the basins.



(a) **(b)**

Figure 11: 11a: The total variation of the difference between μ_b and μ_z as a function of ε^{-1}. 11b: Boxplots representing the variation of the MFPTs for 10 iterations of the method used in 11a, between each pair of basins for $\varepsilon = 1/7$.

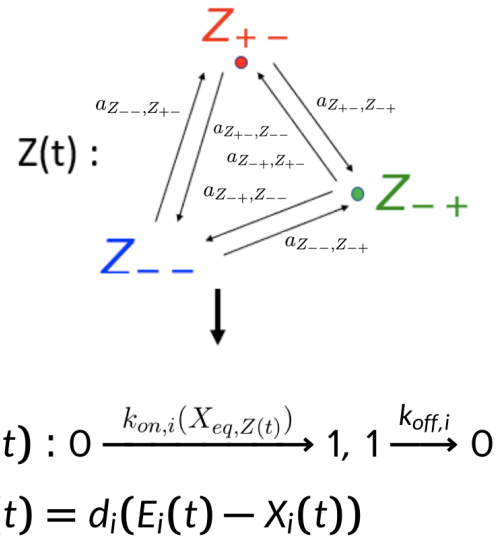


Figure 12: Weak noise approximate model. The Markov chain on the set of basins Z is here illustrated by the one corresponding to the toggle-switch network of Figure 3a.

distribution p of this new model by a mixture of Beta distributions:

$$p \approx \sum_{Z_j \in Z} \mu_z(Z_j) \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right), \tag{28}$$

where μ_z is the stationary distribution of the Markov chain characterizing the coarse-grained model.
In that point of view, the marginal distribution on proteins of a single cell X is characterized by a hidden Markov model: in each basin Z_j, which corresponds to the hidden variable, the vector X is randomly chosen under the quasistationary distribution p_{Z_j} of the reduced process $(E, X \mid Z_j)$. This simplified model provides a useful analytical link between the protein distribution of the PDMP system (depending on the whole GRN) and the coarse-grained model parameters.

This mixture also provides a new approximation for the potential of the system, extending the local potential presented in Section 1.4.4, which is defined on Ω:

$$\begin{aligned} V(x) &\approx -\ln\left(\sum_{Z_j \in Z} \mu_z(Z_j) \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right)(x)\right) \\ &\approx -\ln\left(\sup_{Z_j \in Z}\left(\mu_z(Z_j) \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right)(x)\right)\right). \end{aligned} \tag{29}$$

This last approximation allows to retrieve the definition of the local potential (8), when the boundary of the basins are approximated by the leading term in the Beta mixture. It is justified by the fact that for small ε, the Beta distributions are very concentrated around their centers, meaning that for every basin $Z_k \in Z$, $k \neq j$:

$$\forall x \in Z_j, \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_k})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right)(x) \ll \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right)(x).$$

We supposed that $\forall Z_j \in Z$, $\mu_z(Z_j) > 0$: this is a consequence of the more general assumption that the stationary distribution of the PDMP system is positive on the whole gene expression

space, which is necessary for rigorously deriving an analogy of the Boltzman law for the PDMP system (see Section 1.5.2).

The results of the previous Section leads us to consider this mixture model as promising for combining both simplicity and ability to capture the main ingredients of cell differentiation process.

3 Discussion

3.1 Correspondences between velocities and promoters frequency lead to energetic interpretations

The main idea behind the LDP principle for the PDMP system is that a slow dynamics on proteins coupled to the fast Markov chain on promoters rapidly samples the different states of S_E according to some probability measure $\pi = (\pi_e)_{e \in S_E}$. The value $\sum_{e, e_i = 1}^{n} \pi_e$ corresponds then to the parameter of the Bernoulli parameter of the random variable E_i, and can then be interpreted as the frequency of the promoter e_i.
The distribution π itself follows a LDP, which is the point of view of [42]. The work of [43] consists in averaging the flux associated to the transport of each protein over the measure π, in order to build a new expression of this LDP depending on the protein dynamics. Its coupling with an Hamiltonian function through a Fenchel-Legendre transform allows to apply a wide variety of analytical tools to gain insight on the most probable behaviour of the process, conditioned on rare events.
In this Section, we see how correspondences between these different points of view on the LDP highlight the meaning of the Hamiltonian and Lagrangian functions and lead to some energetic interpretations.

3.1.1 Correspondence between velocity and promoters frequency

We denote $\overline{\Omega^v} = \{u \in C(\Omega, \mathbb{R}^n) \mid \forall x \in \Omega, u(x) \in \bigotimes_{i=1}^{n} [-d_i x_i, d_i(1 - x_i)]\}$.
The velocity field of the PDMP system, Φ, is a n-dimensional vector field, function of the random vectors E, X, which can be written for any $i = 1, \cdots, n$:

$$\Phi_i = (1 - E_i) \times v_{off,i}(X_i) + E_i \times v_{on,i}(X_i), \tag{30}$$

with the functions $v_{off,i} : x_i \mapsto -d_i x_i$ and $v_{on,i} : x_i \mapsto d_i(1 - x_i)$ for any $x \in \Omega$.
Let fix the time and consider that the distribution of X is absolutely continuous. We denote for all $x \in \Omega$ and for all $i = 1, \cdots, n$ $\rho_i(x) = \mathbb{E}(E_i \mid X = x)$ the conditional expectation of promoters E knowing proteins X.
As presented in Section 1.2, the quasistationary approximation identifies ρ to the invariant measure of the Markov chain on the promoter states.
For a given conditional expectation of promoters ρ, the vector field
$v : x \mapsto \mathbb{E}(\Phi \mid X = x)$ is defined for all $x \in \Omega$ by:

$$\begin{aligned} \forall i = 1, \cdots, n,\ v_i(x) &= (1 - \rho_i(x)) v_{off,i}(x) + \rho_i(x) v_{on,i}(x) \\ &= d_i(\rho_i(x) - x_i) \in [-d_i x_i, d_i(1 - x_i)]. \end{aligned} \tag{31}$$

Thus, $v \in \overline{\Omega^v}$. Conversely, we can associate to any vector field $v \in \overline{\Omega^v}$ a unique conditional expectation of promoters states knowing proteins, ρ_v, defined by:

$$\forall x \in \Omega,\ \forall i = 1, \cdots, n : \rho_{v,i}(x) = \frac{v_i(x) - v_{off,i}(x)}{d_i} \in [0, 1]. \tag{32}$$

3.1.2 Dynamics associated to a protein field

We explained above the correspondence between any admissible velocity fields $v \in \Omega^v$ and a unique field ρ_v describing a conditional expectation of promoters states, knowing proteins.

Moreover, the proof of Theorem 2 reveals that for any vector field $p : \Omega \mapsto \mathbb{R}^n$, we can define a unique vector field $\alpha_p : \Omega \mapsto (0,1)^n$ by the expression (20).
As presented in Section 1.5.2, we denote $\zeta = (\overline{\zeta_{0_e}})_{e \in S_E}$ and $V = \overline{V_0}$ the leading order terms of the Taylor expansion in ε of $\overline{\zeta}$ and $\overline{V}$, when the distribution of the system at a time t is defined for all $e \in S_E$ by $u_e(x,t) = \overline{\zeta_e}(x,t) e^{-\frac{\overline{V}(x,t)}{\varepsilon}}$.
On the one hand, for all $i = 1, \cdots, n$, $\sum_{e \in S_E, e_i = 1} \zeta_e(x, \nabla V(x,t))$ then represents the first order approximation of the conditional expectation $\mathbb{E}(E_i \mid X = x)$ at time t.
On the other hand, if we denote the gradient field $p(\cdot) = \nabla V(\cdot, t)$ defined on Ω, we recall that ζ can be written for all $x \in \Omega$ of the form $\zeta(x) = \bigotimes_{i=1}^{n} \begin{pmatrix} 1 - \alpha_{p,i}(x) \\ \alpha_{p,i}(x) \end{pmatrix}$. We then obtain:

$$\alpha_{p,i}(x) = \sum_{e \in S_E, e_i = 1} \zeta_e(x, \nabla V(x,t)) \approx \mathbb{E}(E_i \mid X = x).$$

This interpretation of the vector α_p, combined with the relation (32), allows us to state that the vector $v_{\alpha,p} = (d_i(\alpha_{p,i}(x) - x_i))_{i=1,\cdots,n}$ characterizes, in the weak noise limit, the protein dynamics associated to the protein distribution $e^{\frac{-V(\cdot)}{\varepsilon}}$.
The gradient field p can then be understood as a deformation of the deterministic drift, in the weak noise limit. We verify that the velocity field $v_{\alpha,p}$ corresponds to the deterministic limit if and only if $p = 0$, but is different otherwise.

The duality between p and v then corresponds to a duality between two promoters frequencies α_p and ρ_v.
Recall that for all $p \in \mathbb{R}^n$, from (19), we have:

$$H(x,p) = \sum_{i=1}^{n} p_i d_i(\alpha_{p,i}(x) - x_i).$$

The Lagrangian associated to a velocity field v can be written on every $x \in \Omega$ as a function of α_p and ρ_v:

$$\begin{aligned} L(x, v(x)) &= \sum_{i=1}^{n} p_i(x) d_i(\rho_{v,i}(x) - x_i) - \sum_{i=1}^{n} p_i(x) d_i(\alpha_{p,i}(x) - x_i) \\ &= \langle p(x), v(x) - v_{\alpha,p}(x) \rangle, \end{aligned}$$

where $p(x) = \overline{p_v}(x)$ is defined by the expression (21).

We observe that the velocity $v = (d_i(\rho_{v,i}(x) - x_i))_{i=1,\cdots,n}$ associated to a protein field $p = \nabla V$ by the Fenchel-Legendre transform does not correspond to the protein velocity $v_{\alpha,p}$ associated to the distribution $u = e^{-\frac{V}{\varepsilon}}$ in the weak noise limit, except when the Lagrangian vanishes on (x, v). This is an important limit for developing a physical interpretation of the Hamiltonian system in analogy with Newtonian mechanics.
However, the correspondences between promoters states distributions and velocity fields developed above leads us to draw a parallel with some notions of energy.

3.1.3 Energetic interpretation

Following a classical interpretation in Hamiltonian system theory, we introduce a notion of energy associated to a velocity field:

Definition 4. *Let $x \in \Omega$ and $v \in \Omega^v(x)$. The quantity $E_v(x) = H(x, \overline{p_v}(x))$ is called the energy of the velocity field v on x, where $\overline{p_v}(x)$ is defined by the expression (21).*

Interestingly, combining the expression of the Hamiltonian given in Theorem 2 with the expressions (21) and (32), the energy of a velocity v on every $x \in \Omega$ can be rewritten:

$$E_v(x) = \sum_{i=1}^{n} \frac{\sqrt{k_{on,i}(x)k_{off,i}}}{d_i} \left(\frac{\mathbb{E}_{\rho_{v,i}}(|\Phi_i| \mid X = x)}{\sigma(\rho_{v,i})} - \frac{\mathbb{E}_{\overline{\rho}_i}(|\Phi_i| \mid X = x)}{\sigma(\overline{\rho}_i)} \right)$$

where for all $i = 1, \cdots, n$, Φ_i is the random vector defined by the expression (30) conditionally distributed (knowing proteins) with a Bernoulli distribution of parameter $\rho_{v,i}$, and $\sigma(\rho_{v,i}) = \sqrt{\rho_{v,i}(1-\rho_{v,i})}$ denotes its standard distribution.
Finally, we have $\mathbb{E}_{\rho_{v,i}}(|\Phi_i| \mid X = x) = (1-\rho_{v,i})v_{off,i}(x) + \rho_{v,i}v_{on,i}(x)$, and $\overline{\rho}$ denotes the quasistationary distribution described in (3).
Formally, the energy of a promoter distribution can then be decomposed in two terms : a first term describing its velocity in absolute terms, scaled by its standard deviation, and a second term depending on the network. A high energy distribution is characterized by a fast and deterministic dynamics in regards to the quasistationary distribution.
We remark that this notion of energy does not depend on the protein distribution, but only on its promoter frequency ρ_v around a certain location x. Depending on x only through $\rho_v(x)$ (and the functions $k_{on,i}$), it can then be interpreted as a notion of kinetic energy of a cell.

The potential $V = -\ln(\hat{u})$, where $\hat{u}$ is the marginal on proteins of the steady state distribution of the stochastic process, is classically interpreted as a notion of potential energy, depending this time on the protein field, and not directly on the effective promoters frequency.
Apparently, this notion of energy is not related to the one described previously. Once again, the difficulty for linking these two notions of energy comes from the fact that the velocity associated to the "momentum" $p = \nabla V$ does not correspond to the protein dynamics associated to the marginal distribution on proteins $e^{-\frac{V}{\varepsilon}}$.

3.2 Exemple of application for the mixture model

An interesting application for the estimator of the stationary distribution given by the mixture model is about the computation of the potential energy of the system, as defined in the previous section. The potential energy of a population of cells C, can be approximated by the sum of $(V(c,t))_{c \in C}$, $V(c,t)$ corresponding to the opposite of the logarithm of the stationary distribution of the mixture model p evaluated on the protein level x_c of the cell c.
We represent in Figure 13 the evolution of the potential energy of a population of cells during the differentiation process, simulated from the PDMP system associated to the toggle-switch network presented in Appendix C. The population is initially centered on the attractor of the undifferentiated state Z_{--}. We observe that the potential energy reaches a peak before decreasing.
We remark that in [48], the authors have reconstructed what they called the transcriptional uncertainty landscape during cell differentiation for many available single-cell gene expression data sets. This transcriptional uncertainty corresponds to the stationary potential V, approximated for each cell from the exact stationary distribution of an uncoupled system of PDMPs (with a diagonal interaction matrix).
The universality of such feature seems to be revealed in [48]. Although it cannot be formally

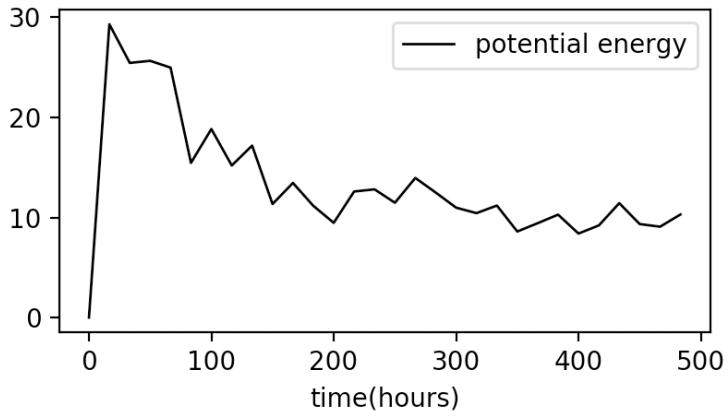


Figure 13: Evolution of the potential energy V of a population of 500 cells along the differentiation process.

linked to intracellular energetic spending yet, we can note that one of the authors recently described a peak in energy consumption during the erythroid differentiation sequence [49].

The mixture model also paves the way for interpreting non-stationary behaviours. Indeed, the mixture distribution can be used as a proxy for non stationary distributions of a PDMP system:

$$p_t \approx \sum_{Z_j \in Z} \mu_{z,t}(Z_j) \prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{\varepsilon d_i}, \frac{k_{off,i}}{\varepsilon d_i}\right).$$

In that case, the only time-dependent parameters are the coordinates of the vector $\mu_{z,t} \in [0,1]^m$ where m is the number of basins, and $\mu_{z,t} = \mu_z$ if t is such that the stationary distribution is reached.
The parameters $(\mu_{z,t}(Z_j), \frac{k_{on,i}(X_{eq,Z_j})}{d_i}, \frac{k_{off,i}}{d_i})_{Z_j \in Z}$ could be inferred from omics data at any time t, for example with an EM algorithm [50], [51].

Conclusion

Reducing a model of gene expression to a discrete coarse-grained model is not a new challenge, ([26], [25]), and it is often hard to perform when the dimension is large. This reduction is closely linked to the notion of landscape through the quasipotential, the analysis of which has been often performed for non mechanistic models, where the random effects are considered as simple noise ([52], [22]), or in small dimension.
In this work, we have proposed a numerical method to approximate the transition rates characterizing the reduction of a mechanistic model of gene expression, for any GRN.
We have built the explicit expression of the Hamilton-Jacobi equation characterizing the quasipotential, for any number of genes, and the explicit expression of the associated variational problem characterizing the landscape. We have deduced for some networks an analytical expression of the energetic costs of switching between the cell types, which approximate the transition rates. These approximations are accurate for a two-dimensional toggle-switch. Testing the accuracy of this method to describe more complex networks would imply to build an approximate solution of the stationary Hamilton-Jacobi equation (22), which will be the subject of future works.
Finally, we have derived from the coarse-grained model a Beta-mixture model able to describe the stationary behavior of a cell in the gene expression space. As far as we know, this is the first time that such an explicit link between a PDMP system describing cell differentiation and a non-Gaussian mixture model is proposed.

Altogether this work establishes a formal basis for the definition of a genetic/epigenetic landscape, given a GRN. It is tempting to now use the same formalism to assess the inverse problem of inferring the most likely GRN, given an (experimentally-determined) cell distribution in the gene expression space, a notoriously difficult task [53], [28].
Such random transitions between cell states has been recently proposed as the basis for facilitating the concomitant maintenance of transcriptional plasticity and stem cell robustness [54]. In this case, the authors have proposed a phenomenological view of the transition dynamics between states. Our work lays the foundation for formally connecting this cellular plasticity to the underlying GRN dynamics.
Finally our work provides the formal basis for the quantitative modelling of stochastic state transitions underlying the generation of diversity in cancer cells ([55], [56]), including the generation of cancer stem cells [57].

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A Tensorial expression of the master equation of the PDMP system

We detail the tensorial expression of the master equation (2) for a two-dimensional network. The general form for the infinitesimal operator can be written:

$$Lu(t,e,x) = \langle F(e,x), \nabla_x u(t,e,x) \rangle + \sum_{e' \in S_E} Q(e,e')(x) u(t,e',x)$$

where F is the vectorial flow associated to the PDMP and Q the matrix associated to the jump operator.
Each jump between two promoters states e, e' can append only between states for which at least one promoter has a different state: in this case, we denote $e \sim e'$.
We have, for any x: $F(e,x) = (d_0(e_0 - x_0), \cdots, d_n(e_n - x_n))^T$. Then, for all $e \in S_E$, the infinitesimal operator can be written:

$$Lu(t,e,x) = \sum_{i=1}^{n} F_i(e,x) \partial_{x_i} u(t,e,x) + \sum_{\{e' | e' \sim e\}} \left(k_{on,i}(x)\delta_{e_i=0} + k_{off,i}\delta_{e_i=1}\right)\left(u(t,e',x) - u(t,e,x)\right).$$

For a two-dimensional process ($n = 2$), there are four possible configurations for the promoter state: $e_{00} = (0,0)$, $e_{01} = (0,1)$, $e_{10} = (1,0)$, $e_{11} = (1,1)$. It is impossible to jump between the states e_{00} and e_{11}. If we denote $u(t,x)$ the four-dimensional vector: $(u_e(t,x))_{e \in S_E}$, we can write the infinitesimal operator in a matrix form:

$$\begin{aligned}
Lu(t,x) = &\underbrace{\begin{pmatrix} -d_1x_1 & 0 & 0 & 0 \\ 0 & -d_1x_1 & 0 & 0 \\ 0 & 0 & d_1(1-x_1) & 0 \\ 0 & 0 & 0 & d_1(1-x_1) \end{pmatrix}}_{F_1(x)} \begin{pmatrix} \partial_{x_1} u_{e_{00}}(t,x) \\ \partial_{x_1} u_{e_{01}}(t,x) \\ \partial_{x_1} u_{e_{10}}(t,x) \\ \partial_{x_1} u_{e_{11}}(t,x) \end{pmatrix} + \\
&\underbrace{\begin{pmatrix} -d_2x_2 & 0 & 0 & 0 \\ 0 & d_2(1-x_2) & 0 & 0 \\ 0 & 0 & -d_2x_2 & 0 \\ 0 & 0 & 0 & d_2(1-x_2) \end{pmatrix}}_{F_2(x)} \begin{pmatrix} \partial_{x_2} u_{e_{00}}(t,x) \\ \partial_{x_2} u_{e_{01}}(t,x) \\ \partial_{x_2} u_{e_{10}}(t,x) \\ \partial_{x_2} u_{e_{11}}(t,x) \end{pmatrix} + \\
&\underbrace{\begin{pmatrix} -k_{on,1}(x) & 0 & k_{on,1}(x) & 0 \\ 0 & -k_{on,1}(x) & 0 & k_{on,1}(x) \\ k_{off,1} & 0 & -k_{off,1} & 0 \\ 0 & k_{off,1} & 0 & -k_{off,1} \end{pmatrix}}_{Q_1(x)} \begin{pmatrix} u_{e_{00}}(t,x) \\ u_{e_{01}}(t,x) \\ u_{e_{10}}(t,x) \\ u_{e_{11}}(t,x) \end{pmatrix} + \\
&\underbrace{\begin{pmatrix} -k_{on,2}(x) & k_{on,2}(x) & 0 & 0 \\ k_{off,2} & -k_{off,2} & 0 & 0 \\ 0 & 0 & -k_{on,2}(x) & k_{on,2}(x) \\ 0 & 0 & k_{off,2} & -k_{off,2} \end{pmatrix}}_{Q_2(x)} \begin{pmatrix} u_{e_{00}}(t,x) \\ u_{e_{01}}(t,x) \\ u_{e_{10}}(t,x) \\ u_{e_{11}}(t,x) \end{pmatrix}.
\end{aligned}$$

We remark that each of these matrices can be written as a tensorial product of the corresponding two-dimensional operator with the identity matrix:

- $F_1(x) = F^{(1)}(x) \otimes I_2$
- $F_2(x) = I_2 \otimes F^{(2)}(x)$
- $Q_1(x) = Q^{(1)}(x) \otimes I_2$
- $Q_2(x) = I_2 \otimes Q^{(2)}(x)$

- $F^{(i)}(x) = \begin{pmatrix} -d_i x_i & 0 \\ 0 & d_i(1-x_i) \end{pmatrix}$ - $Q^{(i)}(x) = \begin{pmatrix} -k_{on,i}(x) & k_{on,i}(x) \\ k_{off,i} & -k_{off,i} \end{pmatrix}$.

The master equation (2) is obtained by taking the adjoint operator of L:

$$\frac{\partial u}{\partial t}(t,x) = L^* u(t,x) = -\sum_{i=1}^{n} \frac{\partial}{\partial x_i}(F_i u)(t,x) + \sum_{i=1}^{n} K_i u(t,x)$$

where $K(x) = Q^T(x)$ is the transpose matrix of Q.

B Example of interaction function

We recall that we keep considering that the vector k_{off} does not depend on the protein vector. The specific interaction function chosen comes from a model of the molecular interactions at the promoter level, described in [28]:

$$k_{on,i}(X) = \frac{k_{0,i} + k_{1,i}(\sigma_i X_i)^{m_{ii}} \Phi_i(X)}{1 + (\sigma_i X_i)^{m_{ii}} \Phi_i(X)} \tag{33}$$

with:

- $k_{0,i}$ the basal rate of expression of gene i,
- $k_{1,i}$ the maximal rate of expression of gene i,
- $m_{i,j}$ an interaction exponent, representing the power of the interaction between genes i and j,
- σ_i is the rescaling factor depending on the parameters of the full model including mRNAs,
- θ a matrix defining the interactions between genes, corresponding to a matrix with diagonal terms defining external stimuli, and
- $\Phi_i(X) = e^{\theta_{i,i}} \prod_{j \neq i} \frac{1+e^{\theta_{j,i}+\theta_{j,j}(\sigma_j X_j)^{m_{ji}}}}{1+e^{\theta_{j,j}(\sigma_j X_j)^{m_{ji}}}}$.

For a two symmetric two-dimensional network, we have for any $x = (x_1, x_2) \in \Omega$:

$$\frac{\partial_{x_2} k_{on,1}}{x_1}(x) = \frac{m_{21} e^{\theta_{22}} x_2^{m21-1} e^{\theta_{11}} x_1^{m11-1}(1-e^{\theta_{12}})}{1 + e^{\theta_{22}} x_2^{m21} + e^{\theta_{11}} x_1^{m11} + x_2^{m21} x_1^{m11} e^{\theta_{11}+\theta_{22}+\theta_{12}}}.$$

When $m_{11} = m_{22} = m_{12} = m_{21}$ and $\theta_{12} = \theta_{21}$, we have then for every $x \in \Omega$:

$$\frac{\partial_{x_2} k_{on,1}}{x_1}(x) = \frac{\partial_{x_1} k_{on,2}}{x_2}(x).$$

Thus, for all $x \in \Omega$ we have:

$$\partial_{x_2}\left(-\frac{k_{on,1}(x)}{d_1 x_1} + \frac{k_{off,1}}{d_1(1-x_1)}\right) = \partial_{x_1}\left(-\frac{k_{on,2}(x)}{d_2 x_2} + \frac{k_{off,2}}{d_2(1-x_2)}\right).$$

By the Poincaré lemma, there exists a function $V \in C^1(\Omega, \mathbb{R})$ which satisfies the condition (23):

$$\forall i = 1,2 : \partial_{x_i} V(x) = -\frac{k_{on,i}(x)}{d_i x_i} + \frac{k_{off,i}}{d_i(1-x_i)}.$$

C Description of the toggle-switch network

This table describes the parameters of the symmetric two-dimensional toggle-switch used all along the article.

(i,j)	$k_{0,i}$	$k_{1,i}$	d_i	σ_i	$m_{i,i}$	$m_{i,j}$	$\theta_{i,i}$	$\theta_{i,j}$	$k_{off,i}$
(1,2)	$0,012/\varepsilon$	$0.39/\varepsilon$	$0,2$	5	3	3	7	-7	$1,25/\varepsilon$
(2,1)	$0,012/\varepsilon$	$0.39/\varepsilon$	$0,2$	5	3	3	7	-7	$1,25/\varepsilon$

D AMS algorithm

We use an adaptive multilevel splitting algorithm described in [38], which provides for every Borel sets (A,B) an unbiased estimator of the probability:

$$\mathbb{P}_x^\varepsilon(\tau_A^\varepsilon < \tau_B^\varepsilon).$$

It is supposed that the random process attains easily A from x, more often than B, called the target set.
The crucial ingredient we need to introduce is a score function $\xi(\cdot)$ able to quantify the adaptive levels describing how close we are from the objective set B from any point x. The variance of the algorithm strongly depends on the choice of this function.
The optimal score function is obviously the function $\mathbb{P}_x^\varepsilon(\tau_A^\varepsilon < \tau_B^\varepsilon)$ itself, which is unknown, but it is proved, at least for Multivel Splitting algorithms applied to stochastic differential equations in [58], [59], that if a certain scalar multiplied by the score function is solution of the stationary HJE coming from the Large deviations setting, the number of simulation needed by the algorithm for keeping the probability in a fixed interval confidence grows sub-exponentially in ε.
In our case, for every basin $Z_j \in Z$, we are interested in computing probabilities substituting A to γ_j and B to another basin Z_k, $k \neq j$. Using the approximation of V given by the expression (29), we obtain the following score function up to a specific constant in each basin:

$$\xi(x) = -\ln\left(\sup_{Z_j \in Z}\left(\prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{d_i}, \frac{k_{off,i}}{d_i}\right)(x)\right)\right).$$

We modify the score function to be adapted for the study of the transitions to each basin Z_j:

$$\xi_j(x) = -\ln\left(\sup_{Zk \in Z}\left(\prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_k})}{d_i}, \frac{k_{off,i}}{d_i}\right)(x)\right)\right) + \ln\left(\prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_j})}{d_i}, \frac{k_{off,i}}{d_i}\right)(x)\right).$$

This function is specific to each basin Z_j but defined in the whole gene expression space. We verify: $\xi_j(x) \leq 0$ if $x \in \Omega \setminus Z_j$ and $\xi_j(x) = 0$ if $x \in Z_j$. We use ξ_j as the score function for the AMS algorithm.

For computing probabilities such that $\mathbb{P}_x^\varepsilon(\tau_{Z_k}^\varepsilon < \tau_{\gamma_j}^\varepsilon)$ for $x \in Z_j$, we need to approximate the boundaries of the basins of attraction, which are unknown. For this sake, we use again the approximate potential function $\xi \approx V$ to approximate the basins. For every $x \in \Omega$, we consider that the associated basin is:

$$Z_j = \underset{Z_k \in Z}{\text{argmax}}\left(\prod_i \text{Beta}\left(\frac{k_{on,i}(X_{eq,Z_k})}{d_i}, \frac{k_{off,i}}{d_i}\right)(x)\right).$$

We use exactly the Adaptative Multilevel Splitting algorithm described in Section 4 of [38]. Two improvements have been taken into account to adapt this algorithm:

• First, a random path associated to the PDMP system does not depend only on the protein state but is characterized at each time t by the 2n-dimensional vector: (X_t, E_t). For any simulated random path, we then need to associate an initial promoter state. However, we know that in the weak noise limit, for a protein state close to the attractor of a basin, the promoter states are rapidly going to be sampled by the quasistationary distribution: heuristically, this initial promoter state will not affect the algorithm. We decide to choose it stochastically under the quasistationary distribution. For every $x_0 \in \Gamma_{min,j}$ beginning a random path in a basin Z_j, we choose for the promoter state of any gene i, e_{i_0}, following a Bernoulli distribution:

$$e_{i_0} \sim B\left(1, \frac{k_{on,i}(x_0)}{k_{off,i} + k_{on,i}(x_0)}\right).$$

• Compared with [38], an advanced algorithm is used to improve the sampling of the entrance time in a set $\gamma_{min,j}$. In practice timestepping is required to approximate the protein dynamics, and it may happen that the exact solution enters $\gamma_{min,j}$ between two time steps, whereas the discrete-time approximation remains outside $\gamma_{min,j}$. We propose a variant of the algorithm studied in [60] for diffusion processes, where a Brownian Bridge approximation gives a more accurate way to test entrance in the set $\gamma_{min,j}$.
In the case of the PDMP system, we replace the Brownian Bridge approximation, by the solution of the ODE describing the protein dynamics: considering that the promoter state e remains constant between two timepoints, the protein concentration of every gene i, $x_i(t)$ is solution of the ODE: $\dot{x_i}(t) = d_i(e_i - x_i(t))$, which implies:

$$\forall t \in [0, \Delta t] : x_i(t) = e_i + (x_i(0) - e_i)e^{-td_i}.$$

We show that for one gene, the problem can be easily solved. Indeed, the function: $f_i(t) = (x_i(t) - X_{i_{eq,Z_j}})^2$ is differentiable and its derivative

$$f_i'(t) = -2d_i(x_i(0) - e_i)e^{-td_i}((e_i - X_{i_{eq,Z_j}}) + (x_i(0) - e_i)e^{-td_i}),$$

vanishes if and only if $(x_i(0) - e_i)e^{-td_i} = (X_{i_{eq,Z_j}} - e_i)$, i.e when

$$t = \frac{1}{d_i} \ln\left(\frac{X_{i_{eq,Z_j}} - e_i}{x_i(0) - e_i}\right) = c_i.$$

Then, if $c_i \leq 0$ or $c_i \geq \Delta t$, the minimum of the squared euclidean distance of the i-th coordinate of the path to the attractor is reached at one of of the points $x_i(0)$ or $x_i(\Delta t)$. If $0 \leq c_i \leq \Delta t$, the extremum is reached at $x_i(c_i)$. This value, if it is a minimum, allows us to determine if the process has reached any neighborhood of an attractor X_{eq,Z_j} between two timepoints.

For more than one gene, the minimum of the sum: $|| x - X_{eq,Z_j} ||^2 = \sum_{i=1}^{n} (x_i(t) - X_{i_{eq,Z_j}})^2$ is more complicated to find. If for all $i = 1, \cdots, n$, $d_i = d$, which is the case of the two-dimensional toggle-switch studied in Section 2.5, the extremum can be explicitly computed:

$$t = \frac{1}{d} \ln\left(\frac{\sum_{i=1}^{n} (X_{i_{eq,Z_j}} - e_i)(x_i(0) - e_i)}{\sum_{i=1}^{n} (x_i(0) - e_i)^2}\right) = c.$$

But we recall that for more than one gene, the set of interest is the isocommitor surface $\gamma_{min,j}$ and not a neighborhood γ_j. An approximation consists in identifying $\gamma_{min,j}$ to the

r-neighborhood of X_{eq,Z_j}, where r is the mean value of $|| x - X_{eq,Z_j} ||^2$ for $x \in \gamma_{min,j}$.
If the parameters d_i are not all similar, we consider that the minimum is close to the minimum for each gene. In this case, it is enough to verify the value of the minimum $x_i(c_i)$ for every gene to know if the process has reached any neighborhood of the attractor between the two timepoints.

E Proofs of Theorems 4 and 5

Proof of Theorem 4. We introduce three lemmas which are necessary to prove this theorem.

First, we state that optimal trajectories associated to a solution of the stationary Hamilton-Jacobi equation cannot cross with the same velocity:

Lemma 2. *Let V_1 and V_2 be two solutions of (22) in $C^1(\Omega, \mathbb{R})$.*
For any trajectories $\phi_t^1, \phi_t^2 \in C_{0T}^{1,pw}(\Omega)$ solutions of the system (16) associated respectively to V_1 and V_2, if there exists $t \in [0,T)$ such that $\phi^1(t) = \phi^2(t)$ and $\dot{\phi}^1(t) = \dot{\phi}^2(t)$, then one has $\phi^1(t) = \phi^2(t)$ for all $t \in [0,T]$.

This can be simply proved by the theorem of characteristics applied to Hamilton-Jacobi equations, which ensures that on these trajectories, we have necessarily for any time t: $\dot{p_i}(t) = -D_{x_i} H(\hat{\phi}(t), p(t))$ (see for example [61]).

A simple consequence of the correspondence between any velocity field of Ω^v and a unique vector field p, proved in Theorem 3, combined with the previous Lemma 2 and applied to the deterministic trajectories, allows us to state:

Corollary 1. *For any solution $V \in C^1(\Omega, \mathbb{R})$ of (22), any trajectory $\phi_t \in C_{0T}^{1,pw}(\Omega)$ solution of the system (16), we have the equivalence:*

$$\begin{aligned} &\exists t \in [0,T),\ \forall i \in \{1, \cdots, n\} :\ \dot{\phi}_i(t) = d_i \left(\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t)) + k_{off,i}} - \phi_i(t) \right) \\ \iff &\forall t \in [0,T) :\ \nabla V(\phi(t)) = 0. \end{aligned}$$

Finally, the following lemma is important for the two first items of the proof:

Lemma 3. *$\forall i \in \{1, \cdots, n\}$, $\forall x \in \Omega$ we have:*

$$\frac{\partial}{\partial p_i} H(x,p) = 0 \iff H_i(x, p_i) = \min_{p_i' \in \mathbb{R}} H_i(x, p_i') \leq 0.$$

Moreover, $\min_{p_i' \in \mathbb{R}} H_i(x, p_i') = 0$ if and only if $x_i = \frac{k_{on,i}(x)}{k_{on,i}(x) + k_{off,i}}$.

Proof. We have seen in the proof of Theorem 3 that for all $i = 1, \cdots, n$ and for all $x \in \Omega$, $H_i(x, \cdot)$ is convex. For any $p_i \in \mathbb{R}$, we have: $H_i(x, p_i) \leq 0 \iff p_i \in [p_{i_1}, p_{i_2}]$ where $p_{i_1} = 0$ and $p_{i_2} = -\frac{k_{on,i}(x)}{d_i x_i} + \frac{k_{off,i}(x)}{d_i(1-x_i)}$. Then, $\frac{\partial}{\partial p_i} H_i(x, p_i) = 0$ on a unique global minimum $\overline{p_i}$ and $H_i(x, \overline{p_i}) \leq 0$.
Moreover, $H_i(x, \overline{p_i}) = 0 \iff p_{i_1} = p_{i_2} = 0$. □

Proof of (i). As a consequence of the Fenchel-Legendre expression of the Lagrangian with $H = 0$ on Ω, for such trajectory ϕ_t we have for any time t:

$$L(\phi(t), \dot{\phi}(t)) = \sum_{i=1}^{n} \partial_{x_i} V(\phi(t)) \dot{\phi}_i(t). \tag{34}$$

Recall that from Theorem 3 :

$$L(\phi(t),\dot{\phi}(t))=0 \iff \forall i=1,\cdots,n:\ \dot{\phi}_i(t)=d_i\left(\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t))+k_{off,i}}-\phi_i(t)\right),$$

we have thus:

$$\dot{\phi}(t)=0 \implies \forall i=1,\cdots,n:\ \dot{\phi}_i(t)=d_i\left(\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t))+k_{off,i}}-\phi_i(t)\right).$$

The velocity field then vanishes only at the equilibrium points of the deterministic system. Conversely, we recall that for such trajectory we have for any t:

$$\begin{cases}\dot{\phi}(t)=\frac{\partial H}{\partial p}(\phi(t),\nabla V(\phi(t))),\\ H(\phi(t),\nabla V(\phi(t)))=0.\end{cases}$$

Thus, from Lemma 3, if for all $i=1,\cdots,n$, $\phi_i(t)=\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t))+k_{off,i}}$, we have:

$$\begin{aligned}H(\phi(t),\nabla V(\phi(t)))=0 &\implies H(\phi(t),\nabla V(\phi(t)))=\sum_{i=1}^{n}\min_{p_i\in\mathbb{R}}H_i(\phi(t),p_i)\\ &\implies \frac{\partial H}{\partial p}(\phi(t),\nabla V(\phi(t)))=0.\end{aligned}$$

Then $\dot{\phi}(t)=0$ and the lemma is proved. □

Proof of (ii). For a solution V of the equation (22) satisfying the condition (C), we can prove the equivalence:

$$\left(\forall i\in\{1,\cdots,n\},\ x_i=\frac{k_{on,i}(x)}{k_{on,i}(x)+k_{off,i}}\right)\iff \nabla V(x)=0.$$

From (i), it is equivalent to prove that for any optimal trajectory associated to such solution V, we have for any time t:

$$\dot{\phi}(t)=0 \iff \nabla V(\phi(t))=0.$$

From the corollary 1, if $\nabla V(\phi(t))=0$, the trajectory is a forward trajectory, along which the gradient is uniformly equal to zero. The condition (C) implies that it is reduced to a single point: $\dot{\phi}(t)=0$. Conversely, as for the first item of the proof:

$$\dot{\phi}(t)=0 \implies L(\phi(t),\dot{\phi}(t)) \implies \forall i:\ \dot{\phi}_i(t)=d_i\left(\frac{k_{on,i}(\phi(t))}{k_{on,i}(\phi(t))+k_{off,i}}-\phi_i(t)\right).$$

We recognize the equation of a forward trajectory, which implies: $\nabla V(\phi(t))=0$.
Thus, for a solution of (22), the condition (C) is equivalent to (C'). □

Proof of (iii). From the relation (34) and the two previous items (i) and (ii), we have on every trajectory ϕ_t associated to a solution V of (22) verifying the condition (C):

$$\dot{\phi}(t)\neq 0 \iff \sum_{i=1}^{n}\partial_{x_i}V(\phi(t))\dot{\phi}_i(t)>0.$$

As $\dot{\phi}(t)\neq 0$ on such trajectories, the term on the right side, equal to $\frac{\partial V}{\partial t}(\phi(t))$, is positive and V strictly increases.
Furthermore, on any forward trajectory $\phi_f(t)$, we have from (15) :

$$0=\int_{T_1}^{T_2}L(\phi_f(t),\dot{\phi}_f(t))dt\geq V(\phi_f(T_2))-V(\phi_f(T_1)).$$

From the corollary 1, the equality holds only for constant solutions $\nabla V = 0$, which are excluded by condition (C). Then $V(\phi(T_1)) < V(\phi(T_2))$.
By definition, for any basin Z_i and for all $x \in Z_i$ there exists a forward trajectory connecting x to the associated attractor X_{eq,Z_i}. So $\forall x \in Z_i$, $V(x) > V(X_{eq,Z_i})$ and X_{eq,Z_i} is a global minimum. □

Proof of (iv). Let V a solution of (22) satisfying the condition (C). We denote by ν_V the velocity field associated to a trajectory ϕ_t solution of the system (16) associated to V: $\dot{\phi}(t) = \nu_V(\phi(t))$. For any basin Z_i, from the previous item (i):

$$\nu_V(X_{eq,Z_i}) = 0 \,\text{and}\, \forall x \in Z_i : \nu_V(x) \neq 0.$$

From (iii), we know that V increases on these trajectories:

$$\forall x \in Z_i : \langle \nabla V(x), \nu_V(x) \rangle > 0.$$

Furthermore: $V(X_{eq,Z_i}) = \min_{x \in Z_i} V(x)$.
We define: $\tilde{V} : x \to V(x) - V(X_{eq,Z_i})$. We have:

$$\forall x \in Z_1 \setminus \{X_{eq,Z_i}\} : \tilde{V}(x) > 0.$$

Thus, $\tilde{V}$ is a Lyapunov function for the system $\dot{\phi}(t) = -\nu_V(\phi(t))$, for which X_{eq,Z_i} is then an asymptotic stable equilibrium.
We deduce that:

$$\forall x \in Z_i, \exists\, \phi_t \in C^{1,pw}(\Omega) : \{\phi(0) = x, \forall t \in [0,T) : \dot{\phi}(t) = -\nu_V(\phi(t)), , \phi(T) \underset{T \to \infty}{\to} X_{eq,Z_i}\}.$$

Reverting time, any point of the basin Z_i can then be reached from any small neighborhood of X_{eq,Z_i}.
Passing to the limit (by continuity of V), we can deduce from Lemma 1 that for any basin Z_i:

$$\forall x \in \overline{Z_i} : Q(X_{eq,Z_i}, x) = V(x) - V(X_{eq,Z_i})$$

As the closure of basins covers the gene expression space, we can conclude that all solutions V are equal up to an additive constant. □

□

Proof of Theorem 5. From the proof of Theorem 4.(iv), we know that:

$$\forall x \in \partial Z_i : Q(X_{eq,Z_i}, x) = V(x) - V(X_{eq,Z_i}), \tag{35}$$

and that for any small neighborhood of X_{eq,Z_i}, there exists a backward trajectory starting inside and converging to x.
Moreover the cost of reaching $\partial\Omega$ is infinite. Indeed, for any velocity field $v \in \Omega^v, x \in \Omega$ and $i = 1, \cdots, n$: $v_i(x) \in [-d_i x_i, d_i(1 - x_i)]$. Then a trajectory which tends to $\partial\Omega$ in a direction i, i.e $x_i \to 0$ or 1, associated to a velocity field v, verifies necessarily $v_i(x) \to 0$. The associated Lagrangian verifies $L(x,v) \geq L_i(x, v_i)$ which tends to $k_{on,i}(x)$ or $k_{off,i}$ when x_i tends respectively to 0 or 1. Recall that for all $i = 1, \cdots, n$, $k_{off,i} > 0$ and $k_{on,i}$ is lower bounded by a positive constant. Thus, the i-th coordinate of the velocity of such trajectory tends to 0 in the direction of $\partial\Omega$ and its Lagrangian is always bigger than a positive constant $k = \min(k_{on,i}(x), k_{off,i})$: the cost of such trajectory is then infinite.
This ensures that for any couple of basins (Z_i, Z_j) such that $\partial Z_i \cap \partial Z_j \neq \varnothing$, the cost of reaching

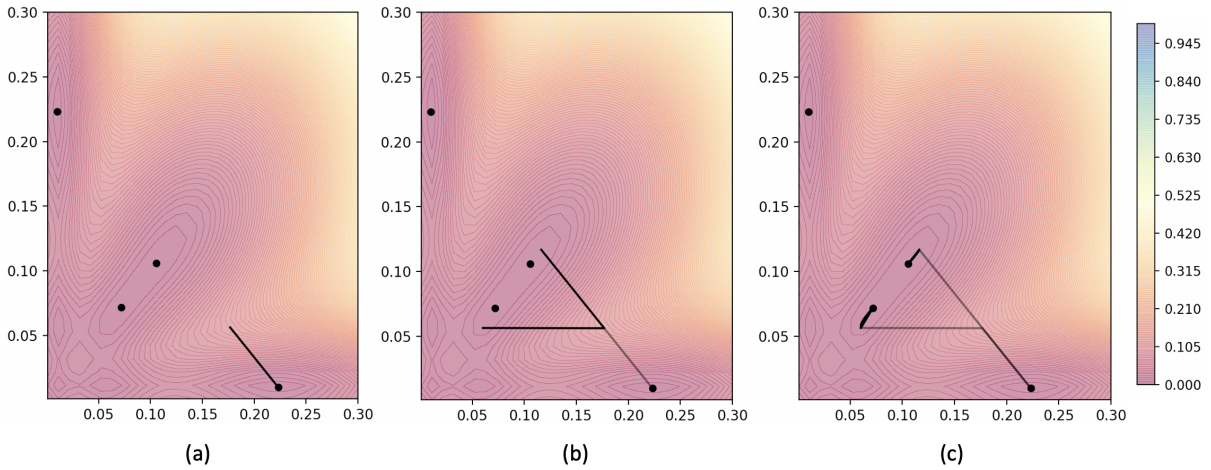

Figure 14: Saddle-point algorithm between two attractors. The color map corresponds to the Lagrangian function associated to the backward trajectories.

any point of $\partial Z_i \cap \partial Z_j$ without crossing ∂Z_i on a point belonging to $R = \bigcup_{k \neq j} \{\partial Z_i \cap \partial Z_k\}$ is given by a trajectory crossing ∂Z_i for the first time on $\partial Z_i \cap \partial Z_j$. Thus, we have:

$$Q_R(X_{eq,Z_i}, \partial Z_i \cap \partial Z_j) = \min_{y \in \partial Z_i \cap \partial Z_j} V(y) - V(X_{eq,Z_i}).$$

From Theorem 4.(iii), V decreases on the forward trajectories: under the hypothesis that any forward trajectory starting in $\partial Z_i \cap \partial Z_j$ stays inside (then it necessarily converges to a saddle point inside), the minimum of V in $\partial Z_i \cap \partial Z_j$ is reached on one (or many) saddle points that we denote y. Since from Theorem 4.(i), the drift of the backward trajectory vanishes on the saddle points, we find that the min of the previous expression is reached on $y \in X_{un,ZiZj}$. □

F Algorithm to find the saddle points

We develop a simple algorithm using the Lagrangian associated to the backward trajectories (25) to find the saddle points of the deterministic system (4). This Lagrangian is a non-negative function which vanishes only at the equilibrium point. Then, if there exists a saddle point connecting two attractors, this function will vanish at this point. Starting on a small neighborhood of the first attractor, we follow the direction of the second one until reaching a maximum on the line (see 14a). Then, we follow different lines, in the direction of each other attractor for which the Lagrangian function decreases (at least, the direction of the second attractor (see 14b)), until reaching a local minimum. We then apply a gradient descent to find a local minimum (see 14c). If this minimum is equal to 0, this is a saddle point, if not we repeat the algorithm from this local minimum until reaching a saddle point or an attractor. Repeating this operation for any ordered couple of attractors $(X_{eq,Z_i}, (X_{eq,Z_j})_{Z_i,Z_j \in Z, i \neq j}$, we are likely to find most of the saddle point of the system. This method is described in pseudo-code in Algorithm 1.

Algorithm 1 Find the list of saddle points: list-saddle-points

Require: • The list of attractors: list-attractors $= (X_{eq,Z_i})_{Z_i \in Z}$
• The Lagrangian function to minimize on the saddle points: $Lag : \mathbb{R}^n \to \mathbb{R}^+$
• A gradient descent function, finding a local minimum of Lag from a point x: gradient-descent(x)
• A subdivision coefficient: $\alpha \ll 1$
while $X_{eq,Z_i} \in$ list-attractors **do**
  $X = X_{eq,Z_i}$
  while $X_{eq,Z_j} \in$ list-attractors$\backslash X_{eq,Z_i}$ **do**
    $Lag0 = Lag(X)$
    $X \leftarrow X + \alpha(X_{eq,Z_i} - X_{eq,Z_j})$
    while $Lag(X) >= Lag0$ **do**
      $Lag0 \leftarrow Lag(X)$
      $X \leftarrow X + \alpha(X_{eq,Z_i} - X_{eq,Z_j})$
    end while
    while $X_{eq,Z_j} \in$ list-attractors$\backslash X_{eq,Z_i}$ **do**
      while $Lag(X) < Lag0$ **do**
        $Lag0 \leftarrow Lag(x)$
        $X \leftarrow X + \alpha(X_{eq,Z_i} - X_{eq,Z_j})$
      end while
      $X_0 =$ gradient-descent(X)
      if $Lag(X_0) = 0$ **then**
        list-saddle-points $\leftarrow X_0$
      end if
    end while
  end while
end while