Analytical and computational study of the stochastic behavior of a chromatin modification circuit*

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***Abstract*— The property of multicellular organisms that allows cells with the same genetic code to maintain distinct identities for the entire life of the organism is known as epigenetic cell memory (ECM). Recently, chromatin modifications and their effect on the DNA structure, that is, the chromatin state, have appeared to have a key role in ECM. Here, we conduct a stochastic analysis of a chromatin modification circuit to determine the effect of time scale separation among key constituent processes on the extent to which the system can keep a stable steady state in the face of noise. Specifically, from the full set of reactions describing the system, we first obtain a reduced circuit model and determine an analytical expression for both the system stationary probability distribution and the switching time between repressed and active chromatin states. Then, we validate these analytical results with stochastic simulations of the original full set of reactions. Our results show that when the basal decay of all chromatin marks is sufficiently slower with respect to the speed of auto and cross-catalysis and of the recruited erasure of all the marks, the stationary distribution shows bimodality, with two concentrated peaks in correspondence of the active and repressed states, but biased towards the repressed state. In accordance with these results, slower basal decay increases the extent of memory of the active and repressed states, suggesting, more broadly, a critical design principle for long-term memory of gene expression states.**

I. Introduction

Epigenetic cell memory (ECM) is the property of multicellular organisms through which cells with the same genetic sequence can keep distinct identities, even under the influence of noise, for the entire organism life. In the last decade, multiple studies have shown how the chromatin state, determined by histone modifications and DNA methylation, has a key role in ECM [1], [2].

This is the reason why in this paper we analyze the dynamics of a chromatin modification circuit proposed in [3], which includes both histone modifications and DNA methylation, with the aim to obtain a mechanistic understanding of the effect of the system's kinetic parameters on the memory of chromatin states. To this end, by exploiting suitable time scale separation among the full set of reactions, we first show that the reaction rate equations lead to a $singular\ singularly\ perturbed\ system$ [4]. This allows us to derive a one-dimensional reduced model convenient for analytical study. In particular, with this reduced model, we construct a one-dimensional Markov chain and derive an expression for the stationary distribution by exploiting detailed balance [5]. We then determine the parameter regime that gives a bimodal distribution with peaks at the active and repressed chromatin states. We further exploit first step analysis [6] to analytically evaluate the extent of memory of the active and repressed state. Finally, we validate the analytical results through a computational study of the original reaction model using Gillespie's Stochastic Simulation Algorithm (SSA) [7].

This paper is organized as follows. In Section 2, we describe the chemical reaction model associated with the chromatin modification circuit and write the corresponding reaction rate equations. Then, in Section 3, we derive a one-dimensional reduced ODE model. In Section 4, we analytically determine the properties of the corresponding Markov chain and validate the obtained results with a computational analysis of the original system of reactions. Concluding remarks and discussion are presented in Section 5.

II. Chromatin modification circuit model

The chromatin modification circuit reaction model considered in this paper, shown in Fig. 1(a), was constructed in [3] based on known molecular interactions from the literature. Here, we give a brief description of the reaction model.

The chromatin modifications considered in the circuit are H3K9 methylation (H3K9me3), H3K4 methylation/acetylation (H3K4me3/ac) and DNA methylation. The basic unit of the model is the nucleosome with DNA wrapped around it, D, that can be modified with H3K4me3/ac, D^A, DNA methylation, D_1^R, H3K9me3, D_2^R, or both H3K9me3 and DNA methylation, D_{12}^R. H3K4 methylation/acetylation are correlated with active chromatin state, enabling gene expression [1]- [8], while

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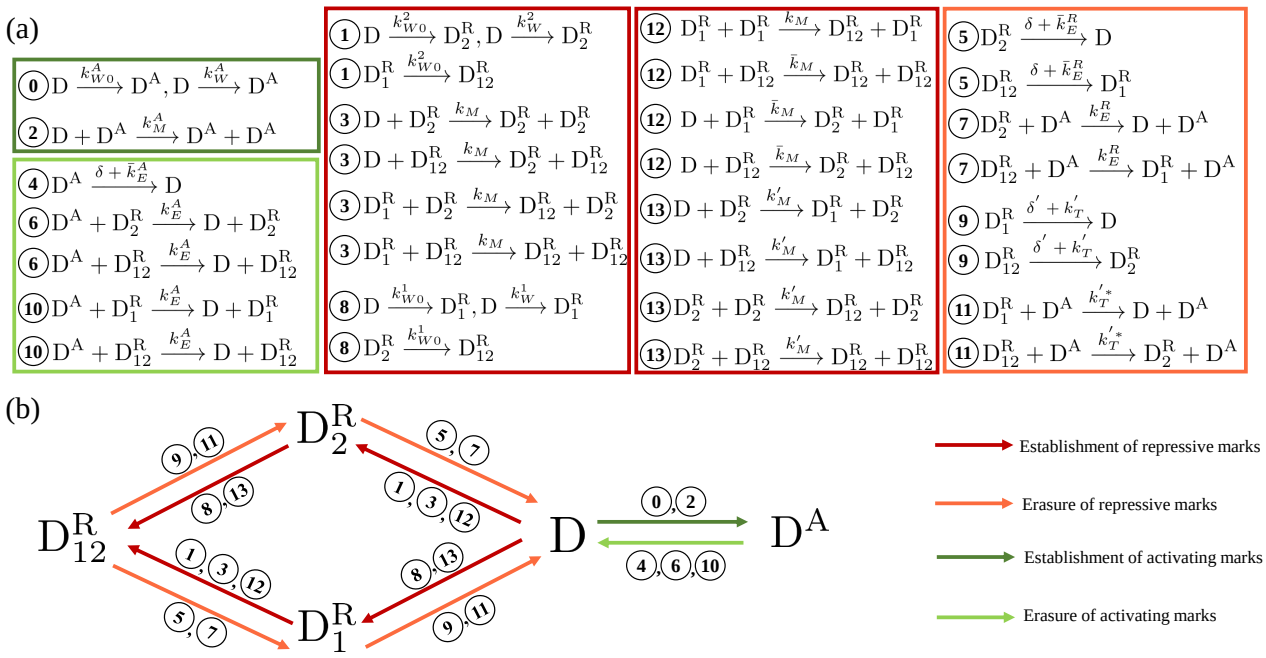

Fig. 1: Reactions and diagram of the chromatin modification circuit. (a) Circuit reaction list. Each reaction is associated with a number. The boxes contain sets of reactions associated with the establishment (dark color) and erasure (light color) of activating (green) and repressive (red) marks, respectively. (b) Chromatin modification circuit diagram. Here, each arrow corresponds to reactions in panel (a) associated with the same number and within the box of the same color.

H3K9me3 and DNA methylation are correlated with repressed chromatin state [9]. The reaction model can be graphically represented by the circuit of Fig. 1(b), whose associated reactions are given in Fig. 1(a). More precisely, the *de novo establishment* of the marks is represented by reactions ⓪, ①, ⑧. Reactions ②, ③ describe the *auto-catalysis* (i.e., a histone mark recruits writer enzymes of the same histone mark to nearby modifiable nucleosomes), while reactions ⑫, ⑬ describe the *cross-catalysis*, wherein DNA methylation and repressive histone modification cooperate by recruiting each other's writer enzymes. Finally, *basal erasure* and *recruited erasure* (i.e., activating and repressive modifications recruit each other's eraser enzymes) are described by reactions ④, ⑤, ⑨ and reactions ⑥, ⑦, ⑩, ⑪, respectively.

Now, defining the number of $\mathrm{D^A}, \mathrm{D_1^R}, \mathrm{D_2^R}, \mathrm{D_{12}^R}, \mathrm{D}$ as n_{D^A}, $n_{D_1^R}$, $n_{D_2^R}$, $n_{D_{12}^R}$ and n_D, let us derive the related ordinary differential equation (ODE) model in terms of the fractions $\bar{D}^A = n_{D^A}/\mathrm{D_{tot}}$, $\bar{D}_1^R = n_{D_1^R}/\mathrm{D_{tot}}$, $\bar{D}_2^R = n_{D_2^R}/\mathrm{D_{tot}}$, $\bar{D}_{12}^R = n_{D_{12}^R}/\mathrm{D_{tot}}$ and $\bar{D} = n_D/\mathrm{D_{tot}}$, with $\mathrm{D_{tot}}$ the total number of nucleosomes on a gene of interest. This can be done by assuming that $\mathrm{D_{tot}}$ is sufficiently large. Now, let us introduce $D_{tot} = \mathrm{D_{tot}}/\Omega$ where Ω is the reaction volume. Then, let us define the normalized inputs: $\bar{u}_1^R = u_{10}^R + u_1^R$, $\bar{u}_2^R = u_{20}^R + u_2^R$ and $\bar{u}^A = u_0^A + u^A$, with $u_{10}^R = k_{W0}^1/k_M^A D_{tot}$, $u_1^R = k_W^1/(k_M^A D_{tot})$, $u_{20}^R = k_{W0}^2/(k_M^A D_{tot})$, $u_2^R = k_W^2/(k_M^A D_{tot})$, $u_0^A = k_{W0}^A/(k_M^A D_{tot})$, and $u^A = k_W^A/(k_M^A D_{tot})$. Furthermore, let us introduce the nondimensional parameters $\alpha = k_M/k_M^A$, $\bar{\alpha} = \bar{k}_M/k_M^A$ and $\alpha' = \frac{k_M'}{k_M^A}$: α is the non-dimensional rate constant associated to auto-catalysis, and $\bar{\alpha}$, α' are the non-dimensional rate constants associated to cross-catalysis. Without loss of generality, we consider these three parameters to have the same order. Let us also define

$$\varepsilon = \frac{\delta + \bar{k}_E^A}{k_M^A D_{tot}}, \quad \varepsilon' = \frac{k_E^A}{k_M^A}, \quad \mu = \frac{k_E^R}{k_E^A}, \quad \mu' = \frac{k_T'^*}{k_E^A}, \qquad (1)$$

with $b = O(1)$ such that $(\delta + \bar{k}_E^R)/(\delta + \bar{k}_E^A) = b\mu$ and $\beta = O(1)$ such that $(\delta' + k_T')/(\delta + \bar{k}_E^A) = \beta\mu'$. Based on these definitions, μ (μ') encapsulates the asymmetry between the erasure rates of repressive histone modifications (DNA methylation) and activating marks. Furthermore, since $(\delta + \bar{k}_E^R)/(k_M^A D_{tot}) = b\varepsilon\mu$, $(\delta' + k_T')/(k_M^A D_{tot}) = \beta\varepsilon\mu'$, $k_E^R/k_M^A = \mu\varepsilon'$ and $k_T'^*/k_M^A = \mu'\varepsilon'$, ε (ε') is a parameter scaling the ratio between the basal (recruited) erasure rate and the auto or cross-catalysis rate of each modification. Considering the normalized time $\tau = tk_M^A D_{tot}$, we can then write the ODEs associated with the chromatin modification circuit:

$$\frac{d\bar{D}_1^R}{d\tau} = (\bar{u}_1^R + \alpha'(\bar{D}_2^R + \bar{D}_{12}^R))\bar{D} + \mu(b\varepsilon + \varepsilon'\bar{D}^A)\bar{D}_{12}^R$$

$$
\begin{aligned}
&- (u_{20}^R + \alpha(\bar{D}_2^R + \bar{D}_{12}^R) + \bar{\alpha}(\bar{D}_1^R + \bar{D}_{12}^R))\bar{D}_1^R \\
&- (\mu^{'}(\beta\varepsilon + \varepsilon^{'}\bar{D}^A))\bar{D}_1^R \\
\frac{d\bar{D}_2^R}{d\tau} &= (\bar{u}_2^R + \alpha(\bar{D}_2^R + \bar{D}_{12}^R) + \bar{\alpha}(\bar{D}_1^R + \bar{D}_{12}^R))\bar{D} \\
&+ \mu^{'}(\beta\varepsilon + \varepsilon^{'}\bar{D}^A)\bar{D}_{12}^R \\
&- (u_{10}^R + \alpha^{'}(\bar{D}_2^R + \bar{D}_{12}^R) + \mu(b\varepsilon + \varepsilon^{'}\bar{D}^A))\bar{D}_2^R \\
\frac{d\bar{D}_{12}^R}{d\tau} &= (u_{10}^R + \alpha^{'}(\bar{D}_2^R + \bar{D}_{12}^R))\bar{D}_2^R \\
&+ (u_{20}^R + \alpha(\bar{D}_2^R + \bar{D}_{12}^R) + \bar{\alpha}(\bar{D}_1^R + \bar{D}_{12}^R))\bar{D}_1^R \\
&- (\mu'(\beta\varepsilon + \varepsilon^{'}\bar{D}^A) + \mu(b\varepsilon + \varepsilon^{'}\bar{D}^A))\bar{D}_{12}^R \\
\frac{d\bar{D}}{d\tau} &= \mu^{'}(\beta\varepsilon + \varepsilon^{'}\bar{D^A})\bar{D}_1^R + \mu(b\varepsilon + \varepsilon^{'}\bar{D^A})\bar{D}_2^R \\
&+ (\varepsilon + \varepsilon^{'}(\bar{D}_1^R + \bar{D}_{12}^R) + \varepsilon^{'}(\bar{D}_2^R + \bar{D}_{12}^R))\bar{D}^A \\
&- (\bar{u}_2^R + \alpha(\bar{D}_2^R + \bar{D}_{12}^R) + \bar{\alpha}(\bar{D}_1^R + \bar{D}_{12}^R))\bar{D} \\
&- (\bar{u}_1^R + \alpha^{'}(\bar{D}_2^R + \bar{D}_{12}^R) + \bar{u}^A + \bar{D}^A)\bar{D} \\
\frac{d\bar{D}^A}{d\tau} &= (\bar{u}^A + \bar{D}^A)\bar{D} \\
&- (\varepsilon + \varepsilon^{'}(\bar{D}_2^R + \bar{D}_{12}^R) + \varepsilon^{'}(\bar{D}_1^R + \bar{D}_{12}^R))\bar{D}^A,
\end{aligned} \tag{2}
$$

with initial conditions that respect $\bar{D}+\bar{D}^A+\bar{D}_1^R+\bar{D}_2^R+\bar{D}_{12}^R = 1$.

III. Model reduction

Here, we exploit time scale separation among reactions to reduce system (2). More precisely, we consider $\varepsilon = c\varepsilon'$, with $c = O(1)$, and $\varepsilon^{'}$ as a small parameter (i.e., erasure reactions much slower than auto and cross-catalytic reactions). This assumption is supported by experimental data showing that the chromatin modifications natural erasure processes are slow [10]. Before showing that system (2), with ε' as a small parameter, is singular singularly perturbed, we first introduce the theorem used to obtain the reduction [4].

Let us start by defining the integral manifold of a general dynamical system $\frac{dx}{dt} = f(x,t)$, with $x \in \mathbb{R}^n$, as a smooth surface S in $\mathbb{R}^n \times \mathbb{R}$ such that any trajectory of the system with at least one point in common with S lies entirely on S [11]- [12]. Now, given $x \in \mathbb{R}^m$ and $y_2 \in \mathbb{R}^n$, let us consider the system

$$
\begin{aligned}
\varepsilon'\dot{x} &= f_1(x, y_2, t, \varepsilon') \\
\varepsilon'\dot{y}_2 &= f_2(x, y_2, t, \varepsilon')
\end{aligned} \tag{3}
$$

and the matrix

$$
A(x, y_2, t, \varepsilon') = \begin{pmatrix} \frac{\partial f_1}{\partial x} & \frac{\partial f_1}{\partial y_2} \\ \frac{\partial f_2}{\partial x} & \frac{\partial f_2}{\partial y_2} \end{pmatrix} = \begin{pmatrix} f_{1x} & f_{1y_2} \\ f_{2x} & f_{2y_2} \end{pmatrix}. \tag{4}
$$

If $A(x, y_2, t, 0)$ is singular on some subspace of $\mathbb{R}^m \times \mathbb{R}^n \times \mathbb{R}$, we say that system (3) is *singular singularly perturbed* [4]. Then, considering $x \in \mathbb{R}^m$, $t \in \mathbb{R}_{\geq 0}$ and introducing the matrix

$$
B(x, \phi(x,t), t, \varepsilon^{'}) = \frac{\partial f_2(x, \phi(x,t), t, \varepsilon^{'})}{\partial y_2}, \tag{5}
$$

let us introduce conditions **B1-B3** [4]:

B1: $f_2(x, y_2, t, 0) = 0$ has a smooth isolated root $y_2 = \phi(x,t)$;

B2: the matrix $A(x, y_2{=}\phi(x,t), t, \varepsilon'{=}0)$ has an m-dimensional kernel and m corresponding linearly independent eigenvectors, and the matrix $B(x, \phi(x,t), t, 0)$ has n eigenvalues $\lambda_i(x,t)$ with negative real part;

B3: the functions f_1 and f_2 and the matrix A are continuously differentiable $(k+2)$ times, with $k \geq 0$ for some positive ε_0' and ρ in the domain $\mathcal{X} = \{(x, y_2, t, \varepsilon') | \; ||y_2 - \phi(x,t)|| \leq \rho, 0 \leq \varepsilon' \leq \varepsilon_0'\}$.

Let us also rewrite system (3) in terms of the new variables $y_1 = y_2 - \phi(x,t)$:

$$
\begin{aligned}
\varepsilon'\dot{x} &= C(x,t)y_1 + F_1(x, y_1, t) + \varepsilon' X(x, y_1, t, \varepsilon') \\
\varepsilon'\dot{y}_1 &= B(x,t)y_1 + F_2(x, y_1, t) + \varepsilon' Y(x, y_1, t, \varepsilon'),
\end{aligned} \tag{6}
$$

in which

$$
\begin{aligned}
C(x,t) &= f_{1y_2}(x, \phi(x,t), t, 0), \\
B(x,t) &= f_{2y_2}(x, \phi(x,t), t, 0), \\
F_1(x, y_1, t) &= f_1(x, y_1 + \phi(x,t), t, 0) - C(x,t)y_1, \\
F_2(x, y_2, t) &= f_2(x, y_1 + \phi(x,t), t, 0) - B(x,t)y_1, \\
\varepsilon' X(x, y_1, t, \varepsilon') &= f_1(x, y_1 + \phi(x,t), t, \varepsilon') \\
&\quad - f_1(x, y_1 + \phi(x,t), t, 0), \\
\varepsilon' Y(x, y_1, t, \varepsilon') &= f_2(x, y_1 + \phi(x,t), t, \varepsilon') \\
&\quad - f_2(x, y_1 + \phi(x,t), t, 0),
\end{aligned} \tag{7}
$$

with F_i, $i = 1, 2$, such that $||F_i(x, y_1, t)|| = O(||y_1||^2)$ and $\varepsilon^{'-1}F_i(x, \varepsilon' y, t)$ continuous in $\mathcal{X}$ [4]. Now, let us introduce the following theorem:

Theorem 1 (Theorem 7.1 from [4])**.** *If conditions* **B1-B3** *hold, then there exists an ε_1', $0 < \varepsilon_1' < \varepsilon_0'$, such that, for any $\varepsilon' \in (0, \varepsilon_1')$, system (6) has a unique slow integral manifold $y_1 = \varepsilon' h(x, t, \varepsilon')$ exponentially attractive, and the motion along this manifold is described by the equation*

$$
\dot{\bar{x}} = X_1(\bar{x}, t, \varepsilon'), \tag{8}
$$

with $X_1(\bar{x}, t, \varepsilon') = C(\bar{x}, t)h(\bar{x}, t, \varepsilon') + X(\bar{x}, \varepsilon' h, t, \varepsilon') + \varepsilon^{'-1}F_1(\bar{x}, \varepsilon' h, t)$ and the function $h(x, t, \varepsilon')$ is k times continuously differentiable with respect to x and t.

Then, for sufficiently small ε', since the slow integral manifold is exponentially attractive, the behavior of the original system (3) near the integral manifold can be determined by studying the reduced system (8).

Now, before applying this theorem to system (2), let us first introduce $\varepsilon = c\varepsilon^{'}$ and the new time variable $\bar{\tau} = \tau\varepsilon'$:

$$
\begin{aligned}
\varepsilon^{'}\frac{d\bar{D}^A}{d\bar{\tau}} &= (\bar{u}^A+\bar{D}^A)\bar{D}\\
&\quad -\varepsilon^{'}(c+(\bar{D}_1^R+\bar{D}_{12}^R)+(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}^A\\
\varepsilon^{'}\frac{d\bar{D}_{12}^R}{d\bar{\tau}} &= (u_{10}^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}_2^R\\
&\quad +(u_{20}^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}_1^R\\
&\quad -\varepsilon^{'}(\mu(bc+\bar{D^A})+\mu^{'}(\beta c+\bar{D^A}))\bar{D}_{12}^R\\
\varepsilon^{'}\frac{d\bar{D}_1^R}{d\bar{\tau}} &= (\bar{u}_1^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}+\varepsilon^{'}\mu(bc+\bar{D}^A)\bar{D}_{12}^R\\
&\quad -(u_{20}^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}_1^R\\
&\quad -(\varepsilon^{'}\mu^{'}(\beta c+\bar{D^A}))\bar{D}_1^R \qquad (9)\\
\varepsilon^{'}\frac{d\bar{D}_2^R}{d\bar{\tau}} &= (\bar{u}_2^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}\\
&\quad +\varepsilon^{'}\mu^{'}(\beta c+\bar{D^A})\bar{D}_{12}^R\\
&\quad -(u_{10}^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R)+\varepsilon^{'}\mu(bc+\bar{D^A}))\bar{D}_2^R\\
\varepsilon^{'}\frac{d\bar{D}}{d\bar{\tau}} &= \varepsilon^{'}(\mu^{'}(\beta c+\bar{D^A})\bar{D}_1^R+\mu(bc+\bar{D^A})\bar{D}_2^R)\\
&\quad +\varepsilon^{'}(c+(\bar{D}_1^R+\bar{D}_{12}^R)+(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}^A\\
&\quad -(\bar{u}_2^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}\\
&\quad -(\bar{u}_1^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{u}^A+\bar{D}^A)\bar{D}.
\end{aligned}
$$

Let us also define x, y, f_1 and f_2 as follows:

$$
x=\begin{pmatrix}\bar{D}^A\\ \bar{D}_{12}^R\end{pmatrix}, y_2=\begin{pmatrix}\bar{D}_1^R\\ \bar{D}_2^R\\ \bar{D}\end{pmatrix}, f_1=\begin{pmatrix}f_{11}\\ f_{12}\end{pmatrix}, f_2=\begin{pmatrix}f_{21}\\ f_{22}\\ f_{23}\end{pmatrix},
$$

$$
\begin{aligned}
f_{11} &= (\bar{u}^A+\bar{D}^A)\bar{D}\\
&\quad -\varepsilon^{'}(c+(\bar{D}_1^R+\bar{D}_{12}^R)+(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}^A,\\
f_{12} &= (u_{10}^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}_2^R\\
&\quad +(u_{20}^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}_1^R\\
&\quad -\varepsilon^{'}(\mu(bc+\bar{D^A})+\mu^{'}(\beta c+\bar{D^A}))\bar{D}_{12}^R,\\
f_{21} &= (\bar{u}_1^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R))\bar{D}+\varepsilon^{'}\mu(bc+\bar{D}^A)\bar{D}_{12}^R\\
&\quad -(u_{20}^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}_1^R\\
&\quad -(\varepsilon^{'}\mu^{'}(\beta c+\bar{D^A}))\bar{D}_1^R, \qquad (10)\\
f_{22} &= (\bar{u}_2^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}\\
&\quad +\varepsilon^{'}\mu^{'}(\beta c+\bar{D^A})\bar{D}_{12}^R\\
&\quad -((u_{10}^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R))+\varepsilon^{'}\mu(bc+\bar{D^A}))\bar{D}_2^R,\\
f_{23} &= \varepsilon^{'}(\mu^{'}(\beta c+\bar{D^A})\bar{D}_1^R+\mu(bc+\bar{D^A})\bar{D}_2^R)\\
&\quad +\varepsilon^{'}(c+(\bar{D}_1^R+\bar{D}_{12}^R)+(\bar{D}_2^R+\bar{D}_{12}^R)\bar{D}^A\\
&\quad -(\bar{u}_2^R+\alpha(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\bar{D}_1^R+\bar{D}_{12}^R))\bar{D}\\
&\quad -(\bar{u}_1^R+\alpha^{'}(\bar{D}_2^R+\bar{D}_{12}^R)+\bar{u}^A+\bar{D}^A)\bar{D}.
\end{aligned}
$$

Now, it is possible to verify that $\phi(x)=(0,0,0)$, with ϕ defined as done in **B1**, and that matrix A, defined in (4), with $\bar{D}=\bar{D}_1^R=\bar{D}_2^R=0$ and $\varepsilon^{'}=0$ is equal to

$$
A(x)=\begin{pmatrix}0_{2,2} & \bar{A}_{2,3}\\ 0_{3,2} & \bar{A}_{3,3}\end{pmatrix} \qquad (11)
$$

with

$$
\begin{aligned}
\bar{A}_{2,3} &= \begin{pmatrix}0 & 0 & (\bar{u}^A+\bar{D}^A)\\ (u_{20}^R+(\alpha+\bar{\alpha})\bar{D}_{12}^R) & (u_{10}^R+\alpha^{'}\bar{D}_{12}^R) & 0\end{pmatrix},\\
\bar{A}_{3,3} &= \begin{pmatrix}\hat{A}_{2,2} & \hat{A}_{2,1}\\ 0_{1,2} & \hat{A}_{1,1}\end{pmatrix}, \quad \hat{A}_{2,1}=\begin{pmatrix}(\bar{u}_1^R+\alpha^{'}\bar{D}_{12}^R)\\ (\bar{u}_2^R+(\alpha+\bar{\alpha})\bar{D}_{12}^R)\end{pmatrix},\\
\hat{A}_{2,2} &= \begin{pmatrix}-(u_{20}^R+(\alpha+\bar{\alpha})\bar{D}_{12}^R) & 0\\ 0 & -(u_{10}^R+\alpha^{'}\bar{D}_{12}^R)\end{pmatrix},\\
\hat{A}_{1,1} &= \begin{pmatrix}-(\bar{u}^A+\bar{D}^A)-(\bar{u}_1^R+\bar{u}_2^R+(\alpha+\bar{\alpha}+\alpha^{'})\bar{D}_{12}^R)\end{pmatrix}.
\end{aligned}
$$

The matrix (11) is singular, and this implies that the system (9) is singular singularly perturbed [4], [12]. Specifically, matrix A has two zero eigenvalues, with two linearly independent eigenvectors associated to them, and matrix $B=\bar{A}_{3,3}$, with B defined in (5). When no external inputs are applied ($u^A{=}u_1^R{=}u_2^R{=}0$ and then $\bar{u}^A{=}u_0^A$, $\bar{u}_1^R{=}u_{10}^R$, and $\bar{u}_2^R{=}u_{20}^R$), matrix B has three eigenvalues with negative real part if $u_{10}^R, u_{20}^R, u_0^A \geq l$ with $l>0$. We can then apply Theorem 1 to reduce our system. Let us first introduce the new variables $\tilde{D}=\bar{D}/\varepsilon^{'}$, $\tilde{D}_1^R=\bar{D}_1^R/\varepsilon^{'}$ and $\tilde{D}_2^R=\bar{D}_2^R/\varepsilon^{'}$ in (9):

$$
\begin{aligned}
\varepsilon^{'}\frac{d\tilde{D}_1^R}{d\bar{\tau}} &= (\bar{u}_1^R+\alpha^{'}(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R))\tilde{D}+\mu(bc+\bar{D}^A)\bar{D}_{12}^R\\
&\quad -(u_{20}^R+\alpha(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R))\tilde{D}_1^R\\
&\quad -(\varepsilon^{'}\mu^{'}(\beta c+\bar{D^A}))\tilde{D}_1^R\\
\varepsilon^{'}\frac{d\tilde{D}_2^R}{d\bar{\tau}} &= (\bar{u}_2^R+\alpha(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R))\tilde{D}\\
&\quad +\mu^{'}(\beta c+\bar{D^A})\bar{D}_{12}^R\\
&\quad -((u_{10}^R+\alpha^{'}(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R))+\varepsilon^{'}\mu(bc+\bar{D^A}))\tilde{D}_2^R\\
\varepsilon^{'}\frac{d\tilde{D}}{d\bar{\tau}} &= \mu^{'}(\beta c+\bar{D^A})\varepsilon^{'}\tilde{D}_1^R+\mu(bc+\bar{D^A})\varepsilon^{'}\tilde{D}_2^R\\
&\quad +(c+(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R)+(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R))\bar{D}^A \qquad (12)\\
&\quad -(\bar{u}_2^R+\alpha(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R))\tilde{D}\\
&\quad -(\bar{u}_1^R+\alpha^{'}(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R)+\bar{u}^A+\bar{D}^A)\tilde{D}\\
\frac{d\bar{D}_{12}^R}{d\bar{\tau}} &= (u_{10}^R+\alpha^{'}(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R))\tilde{D}_2^R\\
&\quad +(u_{20}^R+\alpha(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R)+\bar{\alpha}(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R))\tilde{D}_1^R\\
&\quad -(\mu(bc+\bar{D^A})+\mu^{'}(\beta c+\bar{D^A}))\bar{D}_{12}^R\\
\frac{d\bar{D}^A}{d\bar{\tau}} &= (\bar{u}^A+\bar{D}^A)\tilde{D}\\
&\quad -(c+(\varepsilon^{'}\tilde{D}_1^R+\bar{D}_{12}^R)+(\varepsilon^{'}\tilde{D}_2^R+\bar{D}_{12}^R))\bar{D}^A.
\end{aligned}
$$

Now, in order to find the slow integral manifold, let us first determine the asymptotic expansion of $\tilde{D}$, $\tilde{D}_1^R$ and $\tilde{D}_2^R$:

$$
\begin{aligned}
\tilde{D} &= h_0(\bar{D}^A,\bar{D}_{12}^R,\varepsilon^{'})\\
&= h_{00}(\bar{D}^A,\bar{D}_{12}^R)+\varepsilon^{'}h_{01}(\bar{D}^A,\bar{D}_{12}^R)+O((\varepsilon^{'})^2),\\
\tilde{D}_1^R &= h_1(\bar{D}^A,\bar{D}_{12}^R,\varepsilon^{'}) \qquad (13)\\
&= h_{10}(\bar{D}^A,\bar{D}_{12}^R)+\varepsilon^{'}h_{11}(\bar{D}^A,\bar{D}_{12}^R)+O((\varepsilon^{'})^2),
\end{aligned}
$$

$$\tilde{D}_2^R = h_2(\bar{D}^A, \bar{D}_{12}^R, \varepsilon^{'}) = h_{20}(\bar{D}^A, \bar{D}_{12}^R) + \varepsilon^{'} h_{21}(\bar{D}^A, \bar{D}_{12}^R) + O((\varepsilon^{'})^2).$$

Substituting (13) in the first three equations of (12), we obtain

$$\begin{aligned}
\varepsilon^{'}\frac{dh_1}{d\bar{\tau}} &= \varepsilon^{'}(\frac{\partial h_1}{\partial \bar{D}^A}\frac{d\bar{D}^A}{d\bar{\tau}} + \frac{\partial h_1}{\partial \bar{D}_{12}^R}\frac{d\bar{D}_{12}^R}{d\bar{\tau}}) \\
&= (\bar{u}_1^R + \alpha^{'}(\varepsilon^{'}h_2 + \bar{D}_{12}^R))h_0 + \mu(bc + \bar{D}^A)\bar{D}_{12}^R \\
&- (u_{20}^R + \alpha(\varepsilon^{'}h_2 + \bar{D}_{12}^R) + \bar{\alpha}(\varepsilon^{'}h_1 + \bar{D}_{12}^R))h_1 \\
&- (\varepsilon^{'}\mu^{'}(\beta c + \bar{D^A}))h_1 \\
\varepsilon^{'}\frac{dh_2}{d\bar{\tau}} &= \varepsilon^{'}(\frac{\partial h_2}{\partial \bar{D}^A}\frac{d\bar{D}^A}{d\bar{\tau}} + \frac{\partial h_2}{\partial \bar{D}_{12}^R}\frac{d\bar{D}_{12}^R}{d\bar{\tau}}) \\
&= (\bar{u}_2^R + \alpha(\varepsilon^{'}h_2 + \bar{D}_{12}^R) + \bar{\alpha}(\varepsilon^{'}h_1 + \bar{D}_{12}^R))h_0 \\
&+ \mu^{'}(\beta c + \bar{D^A})\bar{D}_{12}^R \\
&- (u_{10}^R + \alpha^{'}(\varepsilon^{'}h_2 + \bar{D}_{12}^R) + \varepsilon^{'}\mu(bc + \bar{D^A}))h_2 \\
\varepsilon^{'}\frac{dh_0}{d\bar{\tau}} &= \varepsilon^{'}(\frac{\partial h_0}{\partial \bar{D}^A}\frac{d\bar{D}^A}{d\bar{\tau}} + \frac{\partial h_0}{\partial \bar{D}_{12}^R}\frac{d\bar{D}_{12}^R}{d\bar{\tau}}) \\
&= \mu^{'}(\beta c + \bar{D^A})\varepsilon^{'}h_1 + \mu(bc + \bar{D^A})\varepsilon^{'}h_2 \\
&+ (c + (\varepsilon^{'}h_1 + \bar{D}_{12}^R) + (\varepsilon^{'}h_2 + \bar{D}_{12}^R))\bar{D}^A \\
&- (\bar{u}_2^R + \alpha(\varepsilon^{'}h_2 + \bar{D}_{12}^R) + \bar{\alpha}(\varepsilon^{'}h_1 + \bar{D}_{12}^R))h_0 \\
&- (\bar{u}_1^R + \alpha^{'}(\varepsilon^{'}h_2 + \bar{D}_{12}^R) + \bar{u}^A + \bar{D}^A)h_0.
\end{aligned} \tag{14}$$

Now, in order to determine h_{i0} and h_{i1}, with $i = 0, 1, 2$, we compare the right and left hand side of the equations and we equate the terms multiplied by the same power of $\varepsilon^{'}$. Since $\frac{\partial h_{i0}}{\partial \bar{D}_{12}^R}$ and $\frac{\partial h_{i0}}{\partial \bar{D}^A}$ are bounded for any $i = 0, 1, 2$ ($\varepsilon^{'}\frac{\partial h_{i0}}{\partial \bar{D}_{12}^R}, \varepsilon^{'}\frac{\partial h_{i0}}{\partial \bar{D}^A} \ll 1$ for $\varepsilon^{'}$ sufficiently small), we then obtain

$$\begin{aligned}
h_{00} &= \frac{(c + 2\bar{D}_{12}^R)\bar{D}^A}{\bar{u}_2^R + \alpha\bar{D}_{12}^R + \bar{\alpha}\bar{D}_{12}^R + \bar{u}_1^R + \alpha^{'}\bar{D}_{12}^R + \bar{u}^A + \bar{D}^A} \\
h_{10} &= \frac{(\bar{u}_1^R + \alpha^{'}\bar{D}_{12}^R)h_{00} + \mu\left(bc + \bar{D}^A\right)\bar{D}_{12}^R}{u_{20}^R + (\alpha + \bar{\alpha})\bar{D}_{12}^R} \\
h_{20} &= \frac{(\bar{u}_2^R + \alpha\bar{D}_{12}^R + \bar{\alpha}\bar{D}_{12}^R)h_{00} + \mu^{'}\left(\beta c + \bar{D^A}\right)\bar{D}_{12}^R}{u_{10}^R + \alpha^{'}\bar{D}_{12}^R} \\
h_{01} &= \frac{(\mu(cb + \bar{D}^A)h_{20} + \mu^{'}(\beta c + \bar{D}^A)h_{10})}{\bar{u}_2^R + \alpha\bar{D}_{12}^R + \bar{\alpha}\bar{D}_{12}^R + \bar{u}_1^R + \alpha^{'}\bar{D}_{12}^R + \bar{u}^A + \bar{D}^A} \\
h_{11} &= \frac{(\bar{u}_1^R + \alpha^{'}\bar{D}_{12}^R)h_{01}}{u_{20}^R + (\alpha + \bar{\alpha})\bar{D}_{12}^R} \\
&- \frac{(\alpha h_{20} + \bar{\alpha}h_{10} + \mu^{'}(\beta c + \bar{D}^A))h_{10}}{u_{20}^R + (\alpha + \bar{\alpha})\bar{D}_{12}^R} \\
h_{21} &= \frac{(\bar{u}_2^R + (\alpha + \bar{\alpha})\bar{D}_{12}^R)h_{01} - (\alpha^{'}h_{20}^2 + \mu(bc + \bar{D}^A))h_{20}}{u_{10}^R + \alpha^{'}\bar{D}_{12}^R}.
\end{aligned} \tag{15}$$

Then, by plugging into the last two ODEs of (12) the asymptotic expansion of $\tilde{D}$, $\tilde{D}_1^R$ and $\tilde{D}_2^R$ (13) with the expressions of h_{i0} and h_{i1}, $i = 0, 1, 2$, given in (15), we obtain the reduced system:

$$\begin{aligned}
\frac{d\bar{D}^A}{d\tau} &= -\left(\frac{(\varepsilon + 2\varepsilon^{'}\bar{D}_{12}^R)(\bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R)}{\bar{u}^A + \bar{D}^A + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R}\right)\bar{D}^A \\
&+ \left(\frac{(\mu(b\varepsilon + \varepsilon^{'}\bar{D}^A)\mu^{'}(\beta\varepsilon + \varepsilon^{'}\bar{D^A}))\bar{K}(\bar{u}^A + \bar{D}^A)}{\bar{u}^A + \bar{D}^A + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R}\right)\bar{D}_{12}^R \\
\frac{d\bar{D}_{12}^R}{d\tau} &= +\left(\frac{(\varepsilon + 2\varepsilon^{'}\bar{D}_{12}^R)(\bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R)}{\bar{u}^A + \bar{D}^A + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R}\right)\bar{D}^A \\
&- \left(\frac{(\mu(b\varepsilon + \varepsilon^{'}\bar{D}^A)\mu^{'}(\beta\varepsilon + \varepsilon^{'}\bar{D^A}))\bar{K}(\bar{u}^A + \bar{D}^A)}{\bar{u}^A + \bar{D}^A + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\bar{D}_{12}^R}\right)\bar{D}_{12}^R,
\end{aligned} \tag{16}$$

with $\hat{\alpha} = \alpha + \bar{\alpha} + \alpha^{'}$ and in which we re-introduce the original time variable $\tau = \bar{\tau}/\varepsilon'$. The sum of the two equations written above is equal to zero, implying that $\bar{D}^A + \bar{D}_{12}^R =$ constant. Given the conservation law $\bar{D}^A + \bar{D}_{12}^R + \bar{D} + \bar{D}_1^R + \bar{D}_2^R = 1$ and given that $\bar{D} = \varepsilon'\tilde{D} \approx 0$, $\bar{D}_1^R = \varepsilon'\tilde{D}_1^R \approx 0$ and $\bar{D}_2^R = \varepsilon'\tilde{D}_2^R \approx 0$ for small ε', $\bar{D}^A + \bar{D}_{12}^R$ can be set approximately equal to 1 for sufficiently small values of ε'. Now, if we multiply both sides by $D_{tot}(k_E^A D_{tot})$ and define $\bar{k}_W^A = k_{W0}^A + k_W^A$, $\bar{k}_W^1 = k_{W0}^1 + k_W^1$, $\bar{k}_W^2 = k_{W0}^2 + k_W^2$ and $\hat{k}_M = k_M + \bar{k}_M + k_M^{'}$, system (16) can be rewritten as follows:

$$\begin{aligned}
\dot{D}^A =& \frac{(\delta + \bar{k}_E^R + k_E^R D^A)(\delta^{'} + k_T^{'} + k_T^{'*}D^A)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R} \\
&\cdot (\bar{K}_{dim}(\bar{k}_W^A + k_M^A D^A))D_{12}^R \\
&- \frac{(\delta + \bar{k}_E^A + 2k_E^A D_{12}^R)(\bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R} D^A \\
\dot{D}_{12}^R =& \frac{(\delta + \bar{k}_E^A + 2k_E^A D_{12}^R)(\bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R} D^A \\
&- \frac{(\delta + \bar{k}_E^R + k_E^R D^A)(\delta^{'} + k_T^{'} + k_T^{'*}D^A)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R} \\
&\cdot (\bar{K}_{dim}(\bar{k}_W^A + k_M^A D^A))D_{12}^R,
\end{aligned} \tag{17}$$

with $\bar{K}_{dim} = \frac{1}{k_{W0}^1 + k_M^{'} D_{12}^R} + \frac{1}{k_{W0}^2 + (k_M + \bar{k}_M)D_{12}^R}$. The reduced system can be associated with the following chemical reactions:

$$\mathrm{D^A} \xrightarrow{k_{AR}} \mathrm{D_{12}^R}, \quad \mathrm{D_{12}^R} \xrightarrow{k_{RA}} \mathrm{D^A} \tag{18}$$

with reaction rate coefficients defined as

$$\begin{aligned}
k_{AR} =& \frac{(\delta + \bar{k}_E^A + 2k_E^A D_{12}^R)(\bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R}, \\
k_{RA} =& \frac{(\delta + \bar{k}_E^R + k_E^R D^A)(\delta^{'} + k_T^{'} + k_T^{'*}D^A)}{\bar{k}_W^A + k_M^A D^A + \bar{k}_W^2 + \bar{k}_W^1 + \hat{k}_M D_{12}^R} \\
&\cdot \bar{K}_{dim}(\bar{k}_W^A + k_M^A D^A).
\end{aligned} \tag{19}$$

IV. Analytical results

Since $D_{12}^R + D^A \approx D_{tot}$, we can represent the chemical reaction system (18) with a one-dimensional Markov chain, with the state $x = n_{D_{12}^R} \in [0, \mathrm{D_{tot}}]$. Furthermore, for any state $x \in [0, \mathrm{D_{tot}}]$, we define the

rate λ_x, associated with the transition $x \to x+1$, and the rate γ_x, associated with the transition $x \to x-1$, as follows:

$$\lambda_x = \left(\frac{(\varepsilon + 2\varepsilon' \frac{x}{\mathrm{D_{tot}}})(\bar{k}_W^2 + \bar{k}_W^1 + \frac{\hat{k}_M}{\Omega} x)}{\bar{u}^A + \frac{(\mathrm{D_{tot}}-x)}{\mathrm{D_{tot}}} + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\frac{x}{\mathrm{D_{tot}}}} \right) (\mathrm{D_{tot}} - x),$$
$$\gamma_x = \left(\frac{\mu(b\varepsilon + \varepsilon' \frac{(\mathrm{D_{tot}}-x)}{\mathrm{D_{tot}}})\mu'(\beta\varepsilon + \varepsilon' \frac{(\mathrm{D_{tot}}-x)}{\mathrm{D_{tot}}})}{\bar{u}^A + \frac{(\mathrm{D_{tot}}-x)}{\mathrm{D_{tot}}} + \bar{u}_2^R + \bar{u}_1^R + \hat{\alpha}\frac{x}{\mathrm{D_{tot}}}} \right) \tag{20}$$
$$\cdot \bar{K}_x(\bar{k}_W^A + \frac{k_M^A}{\Omega}(\mathrm{D_{tot}} - x))x.$$

In this section, we use this one-dimensional Markov chain to analytically compute the stationary distribution and the duration of the memory of active or repressed chromatin states as a function of the key parameters. Then, we show *via* simulations that the theoretically predicted trends are mirrored by the original chemical reaction system. Let us start with the stationary probability distribution $\pi(x)$. Given that our Markov chain is irreducible and reversible, we can exploit detailed balance [5] and derive an analytical expression for $\pi(x)$:

$$\pi(x) = \prod_{i=1}^{x} \frac{\lambda_{i-1}}{\gamma_i}\pi(0) = \frac{\prod_{i=1}^{x} \frac{\lambda_{i-1}}{\gamma_i}}{\left(1 + \sum_{j=1}^{\mathrm{D_{tot}}} \left(\prod_{i=1}^{j} \frac{\lambda_{i-1}}{\gamma_i}\right)\right)} \tag{21}$$

for $x \in [1, \mathrm{D_{tot}}]$. Then, assuming that $\varepsilon' \neq 0$ and $\varepsilon \ll 1$, $\prod_{i=1}^{j} \frac{\lambda_{i-1}}{\gamma_i} \ll \prod_{i=1}^{\mathrm{D_{tot}}} \frac{\lambda_{i-1}}{\gamma_i}$, $\forall j \in [1, \mathrm{D_{tot}} - 1]$. This means that, when $\varepsilon \ll 1$, we can approximate $\sum_{j=0}^{\mathrm{D_{tot}}} \pi(j) = 1$ as follows:

$$1 = \sum_{j=0}^{\mathrm{D_{tot}}} \pi(j) = \left[\sum_{j=1}^{\mathrm{D_{tot}}} \left(\prod_{i=1}^{j} \frac{\lambda_{i-1}}{\gamma_i}\right)\right] \pi(0) + \pi(0)$$
$$\approx \prod_{i=1}^{\mathrm{D_{tot}}} \frac{\lambda_{i-1}}{\gamma_i}\pi(0) + \pi(0) = \pi(\mathrm{D_{tot}}) + \pi(0),$$

from which, defining $P = \prod_{i=1}^{\mathrm{D_{tot}}} (\lambda_{i-1})/(\gamma_i)$, we obtain

$$\pi_{\varepsilon \ll 1}(x) \approx \begin{cases} \frac{1}{1+P} & \text{if } x = 0 \\ 0 & \text{if } x \neq 0, \mathrm{D_{tot}} \\ \frac{P}{1+P} & \text{if } x = \mathrm{D_{tot}} \end{cases} \tag{22}$$

with

$$P = \frac{(\bar{u}^A + \bar{u}_1^R + u_2^R + \hat{\alpha})}{(\bar{u}^A + \bar{u}_1^R + u_2^R + 1)} \cdot \frac{\bar{u}_1^R + u_2^R}{\mu\mu' b\beta\varepsilon \bar{K}_{\mathrm{D_{tot}}} \bar{u}^A}$$
$$\cdot \prod_{i=1}^{\mathrm{D_{tot}}-1} \left(\frac{2(\bar{u}_1^R + u_2^R + \hat{\alpha}\frac{i}{\mathrm{D_{tot}}})}{\mu\mu'\varepsilon' \frac{(\mathrm{D_{tot}}-i)}{\mathrm{D_{tot}}} \bar{K}_i(\bar{u}^A + \frac{(\mathrm{D_{tot}}-i)}{\mathrm{D_{tot}}})} \right), \tag{23}$$

in which $\bar{K}_{\mathrm{D_{tot}}} = \frac{1}{u_{10}^R + \alpha'} + \frac{1}{u_{20}^R + (\alpha+\bar{\alpha})}$. From this expression it is possible to notice that, if $\varepsilon \ll 1$, the only states in which $\pi(x)$ is not approximately equal to zero are the fully active state ($x = 0$) and the fully repressed state ($x = \mathrm{D_{tot}}$). This means that the distribution is bimodal, with two modes in $x = 0$ and $x = \mathrm{D_{tot}}$, and the probability to have the system in one of the intermediate states is about zero (Fig. 2). Furthermore, when $\varepsilon \to 0$, $P \to 1$, that is $\pi(\mathrm{D_{tot}}) \to 1$ (Fig. 2). This result is consistent with the structural asymmetry of the chromatin modification circuit towards a repressed chromatin state (Fig. 1(b)). Overall, this analysis suggests that when ε is small, a system starting at $x = \mathrm{D_{tot}}$ or at $x = 0$ has a high probability to remain at that state. This qualitatively indicates that ε has a key role in ECM and, when it is small, the memory of the repressed and active chromatin state lasts a long time.

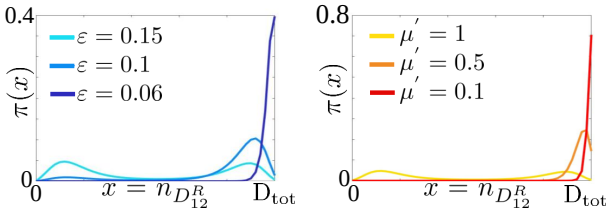

Fig. 2: Effect of ε and μ' on the stationary probability distribution. The stationary distribution $\pi(x)$ obtained analytically, (21), related the circuit reaction model (18). The variable $x = n_{D_{12}^R} \in [0, \mathrm{D_{tot}}]$, with $x = \mathrm{D_{tot}}$ ($x = 0$) corresponding to the fully repressed (active) chromatin state. The parameter values of each panel are listed in Table I.

In order to make this statement mathematically precise, we analytically determine the temporal duration of the memory of the fully repressed and fully active chromatin states. Let us first introduce the definition of *time to memory loss*: given $i, j \in [0, \mathrm{D_{tot}}]$ and defining the hitting time of $x = j$ starting from $x = i$ as $t_i^j := \inf\{t \geq 0 : x(t) = j, \text{ with } x(0) = i\}$, the time to memory loss of the fully repressed chromatin state can be defined as $\tau_{\mathrm{D_{tot}}}^0 = \mathbb{E}(t_{\mathrm{D_{tot}}}^0)$. Similarly, we can define the time to memory loss of the active state as $\tau_0^{\mathrm{D_{tot}}} = \mathbb{E}(t_0^{\mathrm{D_{tot}}})$. Exploiting first step analysis [6], we can compute the time to memory loss of the repressed chromatin state and obtain the following expression:

$$\tau_{\mathrm{D_{tot}}}^0 = \frac{r_{\mathrm{D_{tot}}-1}}{\gamma_{\mathrm{D_{tot}}}} \left(1 + \sum_{x=1}^{\mathrm{D_{tot}}-1} \frac{1}{r_x}\right) + \frac{1}{\gamma_1}$$
$$+ \sum_{x=2}^{\mathrm{D_{tot}}-1} \left[\frac{r_{x-1}}{\gamma_x} \left(1 + \sum_{j=1}^{x-1} \frac{1}{r_j}\right)\right], \tag{24}$$

with $r_x = \frac{\lambda_1\lambda_2\ldots\lambda_x}{\gamma_1\gamma_2\ldots\gamma_x}$ and λ_x and γ_x as defined in (20). Now, normalizing the time with respect to $\frac{k_M^A \mathrm{D_{tot}}}{\Omega}$ ($\bar{\tau}_{\mathrm{D_{tot}}}^0 = \tau_{\mathrm{D_{tot}}}^0 \frac{k_M^A \mathrm{D_{tot}}}{\Omega}$) and assuming $\varepsilon' \neq 0$, when $\varepsilon \ll 1$, the normalized time to memory loss of the repressed state can be approximated as

$$\bar{\tau}_{\mathrm{D_{tot}}}^0 \approx \frac{G_R}{\mu\mu'\varepsilon^2} \left(1 + \sum_{x=1}^{\mathrm{D_{tot}}-1} \frac{G_R^x}{g_1^x(\mu\mu')}\right), \tag{25}$$

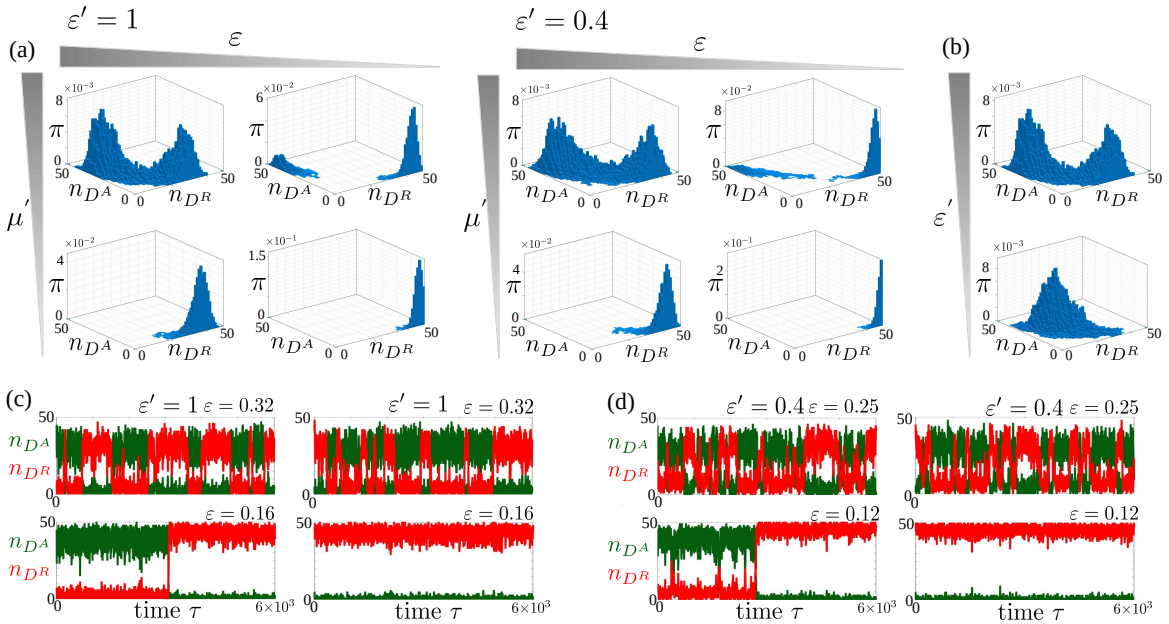

Fig. 3: Computational analysis of the chromatin modification circuit in Fig. 1(a) using stochastic simulation algorithm (SSA). (a) The stationary distribution for the chromatin modification circuit reaction model (Fig. 1(a),(b)). The parameter values are listed in Table II. More precisely, in the left-side plots we have $\varepsilon' = 1$, $\varepsilon = 0.32, 0.16$ and $\mu^{'} = 0.75, 0.4$, and in the right-side plots we have $\varepsilon' = 0.4$, $\varepsilon = 0.25, 0.12$ and $\mu^{'} = 0.75, 0.4$. Here, n_{D^A} and $n_{D^R} = n_{D_1^R} + n_{D_2^R} + n_{D_{12}^R}$ represent the number of nucleosomes with activating and repressive marks, respectively. (b) The stationary distribution for the chromatin modification circuit varying ε'. The parameter values are in Table II. In particular, $\varepsilon = 0.32$ and $\varepsilon' = 1, 0.1$. (c) Time trajectories of n_{D^A} and n_{D^R} starting from the $(n_{D^A}, n_{D^R}) = (50, 0)$, i.e., fully active state, (left-side plots) and $(n_{D^A}, n_{D^R}) = (0, 50)$, i.e., fully repressed state, (right-side plots) for $\varepsilon^{'} = 1$ and different values of ε. (d) Time trajectories of n_{D^A} and n_{D^R}, as described in (b), but with $\varepsilon^{'} = 0.4$. In (c) and (d), the time is normalized ($\tau = t\frac{k_M^A}{\Omega}\mathrm{D_{tot}}$, with Ω representing the reaction volume). The parameter values related to (c) and (d) are listed in Table II.

with g_1^x an increasing function such that $g_1^x(0) = 0$, G_R^x and G_R functions independent of ε, $\mu^{'}$ and μ.. We exploited first step analysis also to determine the time to memory loss of the active chromatin state, obtaining

$$\tau_0^{\mathrm{D_{tot}}} = \frac{\tilde{r}_{\mathrm{D_{tot}}-1}}{\lambda_0}\left(1 + \sum_{x=1}^{\mathrm{D_{tot}}-1}\frac{1}{\tilde{r}_x}\right) + \frac{1}{\lambda_{\mathrm{D_{tot}}-1}} + \sum_{x=2}^{\mathrm{D_{tot}}-1}\left[\frac{\tilde{r}_{x-1}}{\lambda_{\mathrm{D_{tot}}-x}}\left(1 + \sum_{j=1}^{x-1}\frac{1}{\tilde{r}_j}\right)\right], \tag{26}$$

with $\tilde{r}_x = \frac{\gamma_{\mathrm{D_{tot}}-1}\gamma_{\mathrm{D_{tot}}-2}\cdots\gamma_{\mathrm{D_{tot}}-x}}{\lambda_{\mathrm{D_{tot}}-1}\lambda_{\mathrm{D_{tot}}-2}\cdots\lambda_{\mathrm{D_{tot}}-x}}$. Also in this case, normalizing the time ($\bar{\tau}_0^{\mathrm{D_{tot}}} = \tau_0^{\mathrm{D_{tot}}}\frac{k_M^A \mathrm{D_{tot}}}{\Omega}$) and assuming $\varepsilon' \neq 0$, for $\varepsilon \ll 1$ the normalized time to memory loss of the active state can be approximated as

$$\bar{\tau}_0^{\mathrm{D_{tot}}} \approx \frac{G_A}{\varepsilon}\left(1 + \sum_{x=1}^{\mathrm{D_{tot}}-1}\frac{g_2^x(\mu\mu')}{G_A^x}\right), \tag{27}$$

with g_2^x an increasing function such that $g_2^x(0) = 0$ and G_A and G_A^x functions independent of ε, $\mu^{'}$ and μ. By studying the expressions of $\bar{\tau}_{\mathrm{D_{tot}}}^0$ and $\bar{\tau}_0^{\mathrm{D_{tot}}}$, it is possible to notice that decreasing ε leads to higher $\bar{\tau}_{\mathrm{D_{tot}}}^0$ and $\bar{\tau}_0^{\mathrm{D_{tot}}}$. This implies that lower ε extends the memory of both the active and repressed chromatin states. Furthermore, because of the structural asymmetry of the chromatin modification circuit, $\bar{\tau}_{\mathrm{D_{tot}}}^0 = O(1/\varepsilon^2)$ and $\bar{\tau}_0^{\mathrm{D_{tot}}} = O(1/\varepsilon)$, indicating that decreased ε extends the memory of the repressed state much more than the memory of the active state.

Now, let us determine the effect of μ and $\mu^{'}$, the non-dimensional parameters quantifying the asymmmetry between the erasure rates of repressive and activating chromatin modifications, on the cell memory. Studying the expression of the stationary distribution in (22), decreased $\mu^{'}$ or μ lead to higher $\pi_{\ll 1}(\mathrm{D_{tot}})$ and lower $\pi_{\ll 1}(0)$, that is the stationary distribution shifts towards the repressed state (Fig. 2). These results are consistent with the trends with which μ and $\mu^{'}$ affect the time to memory loss, that is, when $\mu^{'}$ or μ decrease, $\bar{\tau}_{\mathrm{D_{tot}}}^0$ increases, while $\bar{\tau}_0^{\mathrm{D_{tot}}}$ decreases.

V. Simulation Results

In order to validate the trends obtained from analytical predictions, which rely on a deterministic quasi-steady state approximation [13], and to demonstrate the validity of these results for a broader parameter range than

Param.	Value left plot	Value right plot	Param.	Value left plot	Value right plot
u_{A0}	0.2	0.2	$\alpha^{'}$	0.2	0.2
u_A	0	0	ϵ	0.15, 0.1, 0.06	0.15
u^1_{R0}	0.2	0.2	$\epsilon^{'}$	0.1	0.1
u^1_R	0	0	b	1	1
u^2_{R0}	0.2	0.2	μ	1	1
u^2_R	0	0	β	1	1
α	0.2	0.2	$\mu^{'}$	1	1, 0.5, 0,1
$\bar{\alpha}$	0.2	0.2			

TABLE I: Parameter values relative to the plots in Fig.2.

Param.	Value (h^{-1}) Fig.3(a) left plots	Value (h^{-1}) Fig.3(a) right plots	Value (h^{-1}) Fig.3(b)	Value (h^{-1}) Fig.3(c)	Value (h^{-1}) Fig.3(d)
k^A_{W0}	3.5	3.5	3.5	3.5	3.5
k^A_W	0	0	0	0	0
k^1_{W0}	3.5	3.5	3.5	3.5	3.5
k^1_W	0	0	0	0	0
k^2_{W0}	3.5	3.5	3.5	3.5	3.5
k^2_W	0	0	0	0	0
k^A_M/Ω	1	1	1	1	1
δ	8,4	6.25,3	8	8,4	6.25,3
$\bar{k}^A_E$	8,4	6.25,3	8	8,4	6.25,3
k^A_E/Ω	1	0.4	1,0.1	1	0.4
k_M/Ω	0.2	0.2	0.2	0.2	0.2
$\bar{k}_M/\Omega$	0.2	0.2	0.2	0.2	0.2
$k^{'}_M/\Omega$	0.2	0.2	0.2	0.2	0.2
$\bar{k}^R_E$	8,4	6.25,3	8	8,4	6.25,3
k^R_E/Ω	1	0.4	1,0.1	1	0.4
$\delta^{'}$	6,3 (up. plots) 3.2,1.6(low. plots)	4.69,2.25 (up.plots) 2.5,1.2(low. plots)	6	6,3	4.69,2.25
$k^{'}_T$	6,3 (up. plots) 3.2,1.6(low. plots)	4.69,2.25 (up.plots) 2.5,1.2(low. plots)	6	6,3	4.69,2.25
$k^{'*}_T/\Omega$	0.75,0.4	0.3,0.16	0.75,0.075	0.75	0.3

TABLE II: Parameter values relative to the plots in Fig.3.

$\varepsilon' \ll 1$ and $\varepsilon = c\varepsilon'$, with $c = O(1)$, we employ the stochastic simulation algorithm (SSA) [7] to computationally study the original chemical reaction system (Fig. 1(a)). The effect of ε and $\mu^{'}$ on the stationary distribution of the original system is in agreement with what we obtained by studying the analytical expressions of $\pi(x)$ provided in (21) (Fig. 3(a)). The parameter $\varepsilon^{'}$ does not significantly alter the effect of ϵ, μ', μ on the distribution. However, decreasing $\varepsilon^{'}$ with respect to ε leads to a bimodal distribution with less concentrated peaks and, by decreasing ε' even further, the distribution becomes unimodal (Fig.3(b)). Furthermore, in the regime in which the system displays a bimodal distribution, the time trajectories show that lower ε leads to a reduction of transitions between the active and repressed chromatin states, especially when starting from the repressed state (Fig. 3(c),(d)), in agreement with analytical findings.

VI. Concluding remarks and discussion

In this work, we considered a circuit including the key interactions between histone modifications and DNA methylation [3] to determine how the system's parameters affect the memory of the active and repressed chromatin states. To this end, we first showed that system (2), with ε' as a small parameter, is singular singularly perturbed and applied a reduction approach proposed in [4]. Then, we analytically determined the stochastic behavior of the reduced system. Finally, we validated and extended these analytical results *via* simulations of the original circuit reaction model (Fig. 1(a)). Our results showed that low ε, and ε' sufficiently larger than ε, lead to a stationary distribution with two concentrated peaks in the active and repressed chromatin states and biased towards the repressed state. This bias can be attributed to the cooperation and coexistence of repressive histone modification and DNA methylation that lead to a structural asymmetry of the chromatin modification circuit towards the repressed state (Fig. 1(b)). Furthermore, low values of $\mu^{'}$ and μ can make this asymmetry even more pronounced (*viceversa* for large $\mu^{'}$ and μ). In agreement with these results, our analysis showed longer memory of the active and repressed states, but more for the repressed state, for smaller values of ε, and longer repressed state memory and shorter active state memory for lower values of $\mu^{'}$ and μ. Future work will investigate how positive and negative transcription factor-mediated autoregulation loops affect the chromatin modification dynamics and thus epigenetic cell memory.

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