

A continuum reaction-diffusion model for spread of gene silencing in chromosomal inactivation (Supplementary Data)

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1 Introduction

X chromosome inactivation (XCI) is a fascinating biological phenomenon that achieves the equalization of X chromosome-linked gene expression between male and female cells. It operates akin to a biochemical switch, deactivating redundant X chromosomes in female cells. Investigating XCI serves as a crucial avenue for comprehending large-scale gene silencing processes. While conventional particle-based statistical modeling has been instrumental in studying XCI, it encounters challenges in addressing certain biochemical aspects, particularly the intricate feedback mechanisms associated with XCI, due to their computational complexity. As a result, this paper presents a continuum model based on partial differential equations for elucidating the gene silencing process.

2 The Model

We have developed a partial differential equation-based reaction diffusion model to study the process of XCI. Using the partial differential equation framework has equipped our study with several advantages, such as implementing reaction kinetics with complex biochemical feedback mechanisms and computational efficiency, to name a few. In contrast, in the initial phase, our study suffered from a striking disadvantage of using a continuum formulation, i.e., incorporating the effect of the intricate three-dimensional architecture of the X chromosome on the process of XCI. Nevertheless, by utilizing the reaction kinetics of X chromosome inactivation, we have managed to include the chromosomal structure into our model, which we will discuss in the later part of this paper. We have developed our model in a modular approach, dividing the process of XCI into two different modules. Once we have standardized each individual module, we combine them to build the complete model. The process of XCI can be divided into two major modules, namely, (1) spatio-temporal dynamics of the key biochemical player of XCI, a long non coding RNA called XIST, and (2) spatio-temporal dynamics of XIST-mediated methylation of the nucleosomes. Once we have settled with both modules through appropriate parameter optimization, we combine them through another module, namely, (3) XIST tethering to the X chromosome. Detailed descriptions of these modules will be provided in the following subsections.

2.1 Spatio-temporal dynamics of XIST molecules (XIST module)

Recent experimental studies reveal that XIST molecules exhibit negative auto-regulation on their own production and positive auto-regulation on their own degradation (Fig.S1A). In our modeling

framework, we have aimed to capture this feedback mechanism by formulating the following partial differential equation originating from mass action kinetics,

$$\frac{\partial[XIST]}{\partial t} = \frac{\beta_X}{1 + \left[\frac{[XIST]}{\phi_\beta}\right]^{n_\beta}} - \frac{\alpha_X[XIST]}{1 + \left[\frac{[XIST]}{\phi_\alpha}\right]^{-n_\alpha}} + D_X \nabla^2[XIST] \quad (\text{S.1})$$

Where, the first and second terms constitute two Hill functions and are representing production, and degradation of XIST molecules respectively. The third term constitutes a Laplacian and it is representing the spatial diffusion of the XIST molecules. Furthermore, β_X is the basal transcription rate of XIST RNA molecules, α_X is the basal degradation rate of the same; ϕ_β and ϕ_α are the negative and positive feedback coefficients, respectively for XIST production and degradation. n_β and n_α describe the corresponding Hill coefficients. We have investigated the effect of feedback mechanisms on the spatio-temporal profile of XIST molecules which we will be discussed in the upcoming sections.

2.2 Spatio-temporal dynamics of chromosomal inactivation (Chromatin module)

During X chromosome inactivation (XCI), the long non-coding RNA XIST spreads throughout the inactive X chromosome in cis, eventually tethering to specific regions of the chromosome's architecture. Once tethered, XIST molecules recruit the Polycomb Repressive Complex 2 (PRC2), a reader-writer enzyme, and transition its state from inactive to active. The activated PRC2 enzyme then initiates gene silencing by methylating the nucleosomes.

Recent experimental findings indicate that the rate of transition of PRC2 from an inactive to an active state is positively regulated by neighboring inactive epigenetic markers, specifically of the type H3K27Me3.

Based on these biological processes, we have developed a set of partial differential equations with embedded bi-stability to describe the spatiotemporal dynamics of the active and inactive

forms of the PRC2 enzyme.

$$\frac{\partial u_1}{\partial t} = f(u_1, u_2) + \beta_{u_1} - \alpha_{u_1} u_1 + f_s([XIST]) + D_{u_1} \nabla^2 u_1 \quad (\text{S.2})$$

$$\frac{\partial u_2}{\partial t} = -f(u_1, u_2) + \beta_{u_2} - \alpha_{u_2} u_2 - f_s([XIST]) + D_{u_2} \nabla^2 u_2 \quad (\text{S.3})$$

$$f(u_1, u_2) = u_2 \left[\kappa_0 + \frac{\gamma u_1^n}{K^n + u_1^n} \right] - \delta u_1 \quad (\text{S.4})$$

$$u_1 = [\text{Active-PRC2}]; u_2 = [\text{PRC2}]$$

Where, u_1 stands for the active form of PRC2 and u_2 the inactive form. As the process of chromosomal inactivation is carried out by active PRC2, hence in the context of our model the higher concentration of u_1 represents the inactivation of chromosome and vice versa. β_i , α_i and D_i portrays respectively the production rate and degradation rate and rate of diffusion of corresponding species. κ_0 is the rate of switching from the inactive to active form and δ is the rate of the reverse switching. γ and K both modulate the positive feedback imparted by the neighboring epigenetic markers with different degree of sensitivity. n is the Hill coefficient. f_s is the stimulation function which is extensively dependent on the spatio-temporal profile of XIST molecules and probability of tethering. The detailed description for the dependency of f_s on the spatio temporal profile of XIST molecules will be discussed in the next subsection where we have addressed the XIST tethering module.

In this context, we would like to discuss the biologically plausible ranges of certain parameters used in our model. The PRC2 complex primarily consists of Polycomb Group (PcG) proteins, including EZH1/2, EED, and SUZ12 [1]. Since experimental data on the overall production rate of PRC2 are currently unavailable, we have assumed that its synthesis rate is correlated with that of PcG proteins and operates within their biologically permissible synthesis range. Reported turnover rates for PcG proteins typically range between 1 and 1000 molecules per hour per cell considering the somatic cells as well as cancerous cells [2, 3]. Assuming homogeneous distribution and typical mammalian cell volume of $\approx 2 \text{ pL}$ [4] leads us to the acceptable range of synthesis rate 0.1-100 pM/sec . Similar calculation reveals the range of degradation rate to be 14-140 pM/sec [5].

For the chromatin module in the model, the diffusion coefficient of active-PRC2 (D_{u_1}) is assumed to be markedly lower than that of its inactive form (D_{u_2}). This assumption is grounded in the biochemical and biophysical understanding that active-PRC2 exhibits more extensive molecular interactions within the chromatin environment compared to inactive PRC2. Structurally, active-PRC2 engages with chromatin and cofactors, forming stable complexes that reduce its mobility. In contrast, inactive PRC2 is largely unbound and diffuses more freely within the nucleoplasm.

Experimental evidence from live-cell single molecule imaging shows that a substantial fraction of PRC2 is rapidly diffusing in the nucleus, representing inactive or less-chromatin-bound states, while the chromatin-bound, active fraction exhibits constrained diffusion due to stable interactions with nucleosomes and regulatory proteins [6, 7]. Mechanistically, active PRC2's higher affinity and longer residence times on chromatin arise from its ability to recognize and methylate specific histone marks, recruit accessory subunits, and form higher-order complexes that modulate gene expression. This extensive network of interactions effectively lowers the complex's diffusion coefficient relative to the inactive, more soluble form, thus justifying the differential diffusion parameters assumed in chromatin modeling frameworks.

2.3 XIST tethering to the X chromosome (Tethering module)

After standardizing the previous modules, we integrate them with a third module called the XIST tethering module to develop a comprehensive model for X Chromosome Inactivation (XCI). Within this module, we have incorporated spatial tethering sites for XIST molecules. The tethering process at a specific spatial point is governed by two key factors. First, the concentration of XIST molecules, which is derived from the simulation of Eq. S.1. Second, the tethering probability, defined as the ratio of the maximum allowed tethering sites (n_{thr}) to the total number of available sites for tethering in the chromosome structure (n_{total}). Therefore, by combining these conditions, we can express the tethering probability (P_{thr}) at any given spatial co-ordinate $\mathbf{q}$ and time point t as:

$$P_{\text{thr}}(\mathbf{q}, t) = \begin{cases} 1 & \text{if } [XIST](\mathbf{q}, t) > [XIST]_C(t) \text{ and } r < \frac{n_{\text{thr}}}{n_{\text{total}}} \\ 0 & \text{otherwise} \end{cases} \quad (\text{S.5})$$

Where, r is a random variable with uniform distribution, spanned between 0 and 1 and $[XIST]_C(t)$ is the time dependent critical concentration for tethering of XIST molecules to the X chromosome and we have defined it as follows:

$$[XIST]_C(t) = [XIST]_{\text{min}}(t) + \phi_{\text{thr}} \{ [XIST]_{\text{max}}(t) - [XIST]_{\text{min}}(t) \} \quad (\text{S.6})$$

where, $[XIST]_{\text{min}}$, and $[XIST]_{\text{max}}$ is the minimum and maximum XIST concentration at time t , respectively. ϕ_{thr} is the tethering coefficient (as the spatial profile of XIST molecules is time dependent so the critical XIST concentration for tethering should also be time dependent) which controls the susceptibility of the XIST molecules for tethering to the chromosome and this parameter should be fine tuned based upon the spatio-temporal profile of XIST molecules. In summary,

the tethering module determines the initial stimulation points for the XCI module by utilizing the simulation results from the XIST module.

3 Methods

We conducted our studies in a manner reminiscent of our model development, where we performed numerical simulations of partial differential equations chronologically. Initially, we focused on the XIST module, conducting several numerical simulations to uncover the determining factors influencing the spatio-temporal profile of XIST molecules. Subsequently, we systematically explored the XCI module to incorporate biologically relevant features and identify determining factors affecting the extent of chromosomal inactivation. Thereafter, we incorporate the tethering module into our simulation and conduct a thorough computational investigation of the entire model. Moreover, we have investigated the effect of chromosomal compaction on the final profile of X chromosome inactivation in the context of our model. Up until this point, we have performed the simulation of our model considering only one spatial dimension to unravel salient features of our model in a phenomenological manner. Finally, we have implemented our model in three spatial dimensions including all the features we have discussed thus far and compared the simulation result with experimental results. In this section we will present a detailed description of several aspects of the simulation methods that we have utilized to obtain the results.

3.1 Simulations performed in one dimension

3.1.1 XIST module

We have performed numerical simulation of the XIST module, i.e., partial differential equations described the Eq.S.1. We have used the explicit Euler algorithm for numerical integration of the PDEs and used central difference formula to evaluate the Laplacian for a particular spatial point at each time steps. The central difference formula for one dimensional Laplacian can be written as:

$$\nabla^2 u(x) = \frac{u(x + \Delta x) + u(x - \Delta x) - 2u(x)}{\Delta x^2} \quad (\text{S.7})$$

Here, u is the arbitrary species of concern and Δx is the spatial grid size.

We simulated the XIST module across various parameter sets to identify the optimal feedback strength for XIST production and degradation (ϕ_β and ϕ_α respectively), ensuring the most rapid and homogeneous spatial spreading of XIST molecules over time. The results are depicted in the

following two figures: A pertinent point to be noted is that for the simulations we have set $\beta = 1$ for X inactive center, i.e., the transcription site of XIST and $\beta = 0$ otherwise. The Figs.S1 and S2 together reveal that there is an optimal value of feedback coefficients (ϕ_β, ϕ_α) for which the XIST spreading takes place most rapidly and most homogeneously. To identify the optimal parameter set we have devised a scheme of bubble plot for the entire parameter scan. For each of the parameter set we have devised a bubble whose radius is proportional to the ratio of minima to the maxima of the XIST concentration throughout space at the asymptotic time limit. Moreover, the brightness of the color of the bubbles were determined by the speed of attainment of the steady value. More precisely, the brightness is directly proportional to the area under the curve depicting the variation of normalized spatially averaged XIST concentration with time. The entire scheme is illustrated in Fig.S3 for enhancing the clarity. This bubble visualization reveals that for $\phi_\alpha = 1.0$ and $\phi_\beta = 0.1$, the XIST molecules spread throughout the space with satisfactory temporal rapidness and spatial homogeneity.

3.1.2 XCI (Chromatin) module

Subsequently, we studied the X chromosome inactivation by numerically simulating the PDEs described by Eqs.(S.2-S.3). Firstly, we performed the numerical continuation of the constituting ODE parts using δ as the free parameter of the PDEs to evaluate the steady states of the system. Then we curated appropriate steady states from the numerical continuation result. The result of the numerical continuation and curated steady states are delineated in Fig.S4. Thereafter, We used numerical techniques to simulate the PDEs associated to XCI module, similar to that of the XIST module. At the initial phase of the simulation, we have stimulated the system out of the steady state to initiate the inactivation process. The functional form of the stimulation can be written as follows:

$$f_s = \frac{s}{2} \sin^8 \left(\frac{n_{\text{stim}} \pi x}{L} \right) \text{ for } (0 < t < t_1) \quad (\text{S.8})$$

$$= \frac{s}{2} \cos \left(\frac{\pi(t - t_1)}{2(t_2 - t_1)} \right) \sin^8 \left(\frac{n_{\text{stim}} \pi x}{L} \right) \text{ for } (t_1 < t < t_2) \quad (\text{S.9})$$

$$= 0 \text{ for } (t_2 < t) \quad (\text{S.10})$$

We used this manual stimulation to conduct a phenomenological study of XCI in one spatial dimension. Later in this paper, the stimuli are governed by the spatio-temporal dynamics of the XIST molecules. Subsequently, using the aforementioned stimulation function, we simulated various scenarios of X chromosome inactivation, revealing numerous interesting features portrayed

Normalized spatio-temporal profile of [XIST]

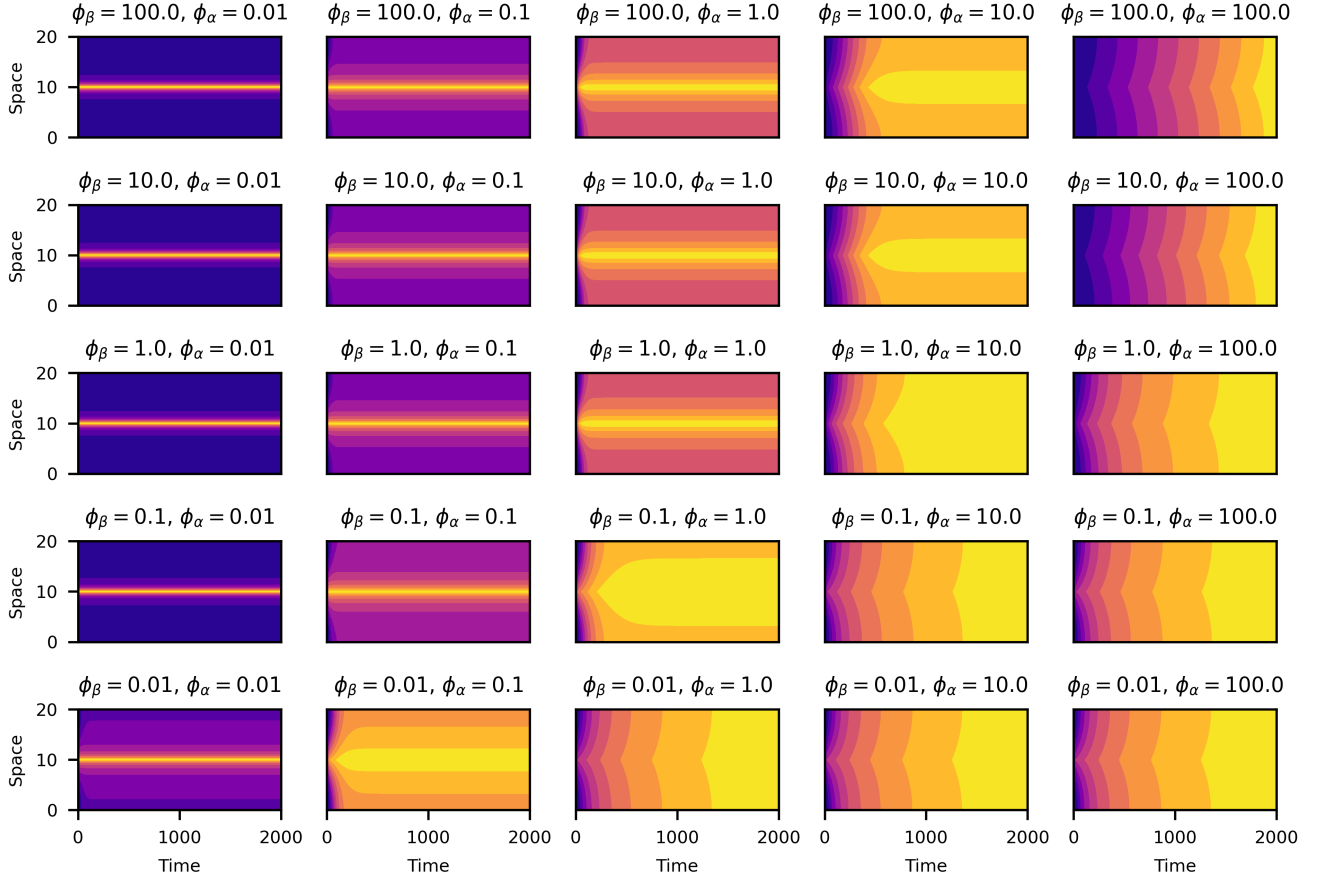


Fig. S1: Spatio-temporal profile of XIST concentration for different values of feedback coefficients of production ϕ_β and of degradation ϕ_α . The simulations are carried out using the following parameter values: $[XIST]_0 = 0$; $\Delta x = 0.1$; $\Delta t = 10^{-3}$; $n_\beta = 4$, $n_\alpha = 4$; $D_X = 1.0$; $\alpha_X = 1.0$; $\beta_X = 1.0$ (units arbitrary).

Spatial profile of XIST molecules at final time

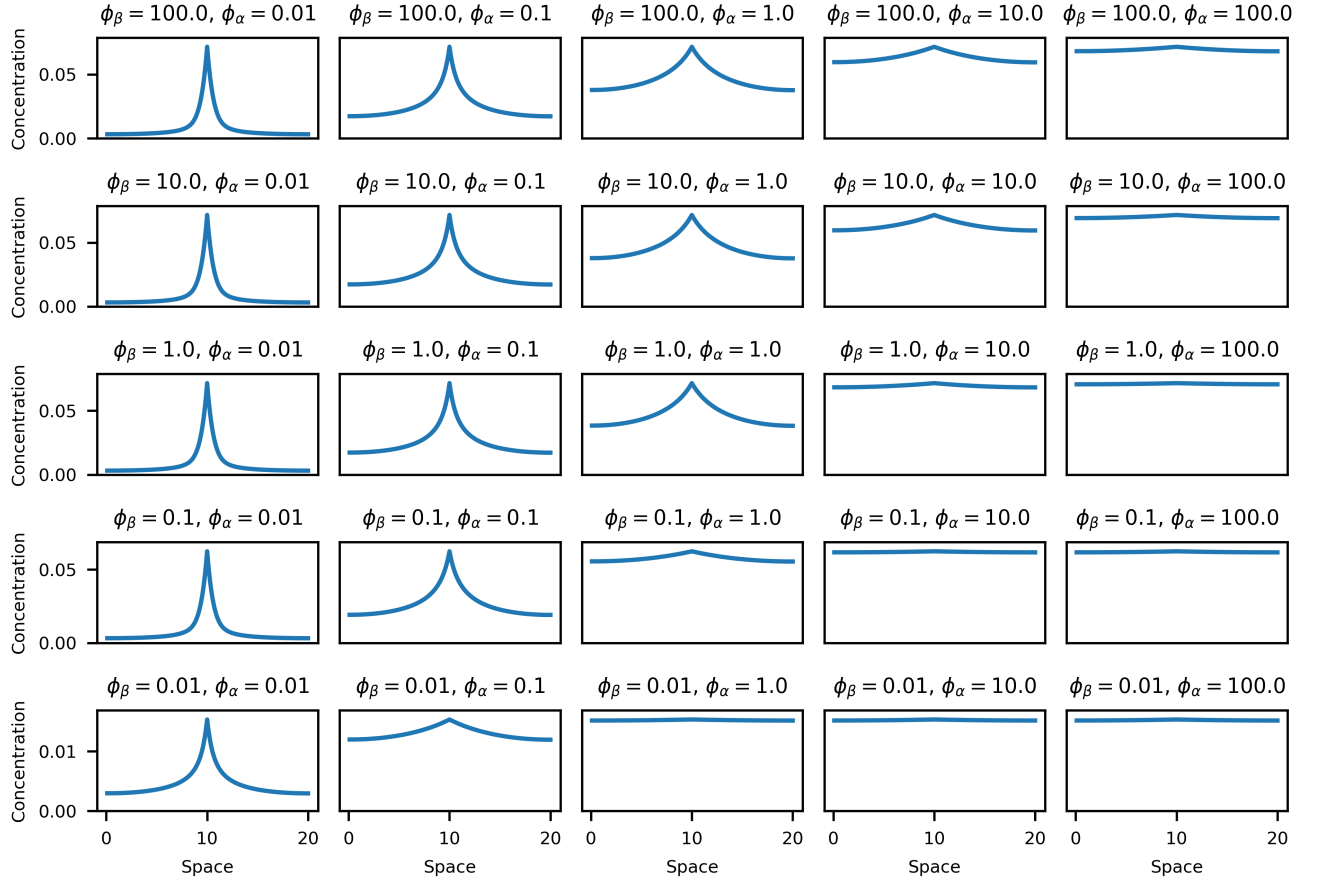


Fig. S2: Steady spatial profile of XIST concentration at $t=2000$ time units for different values of feedback coefficients of production ϕ_β and of degradation ϕ_α . The simulations are carried out using the parameter values used for Fig.S1

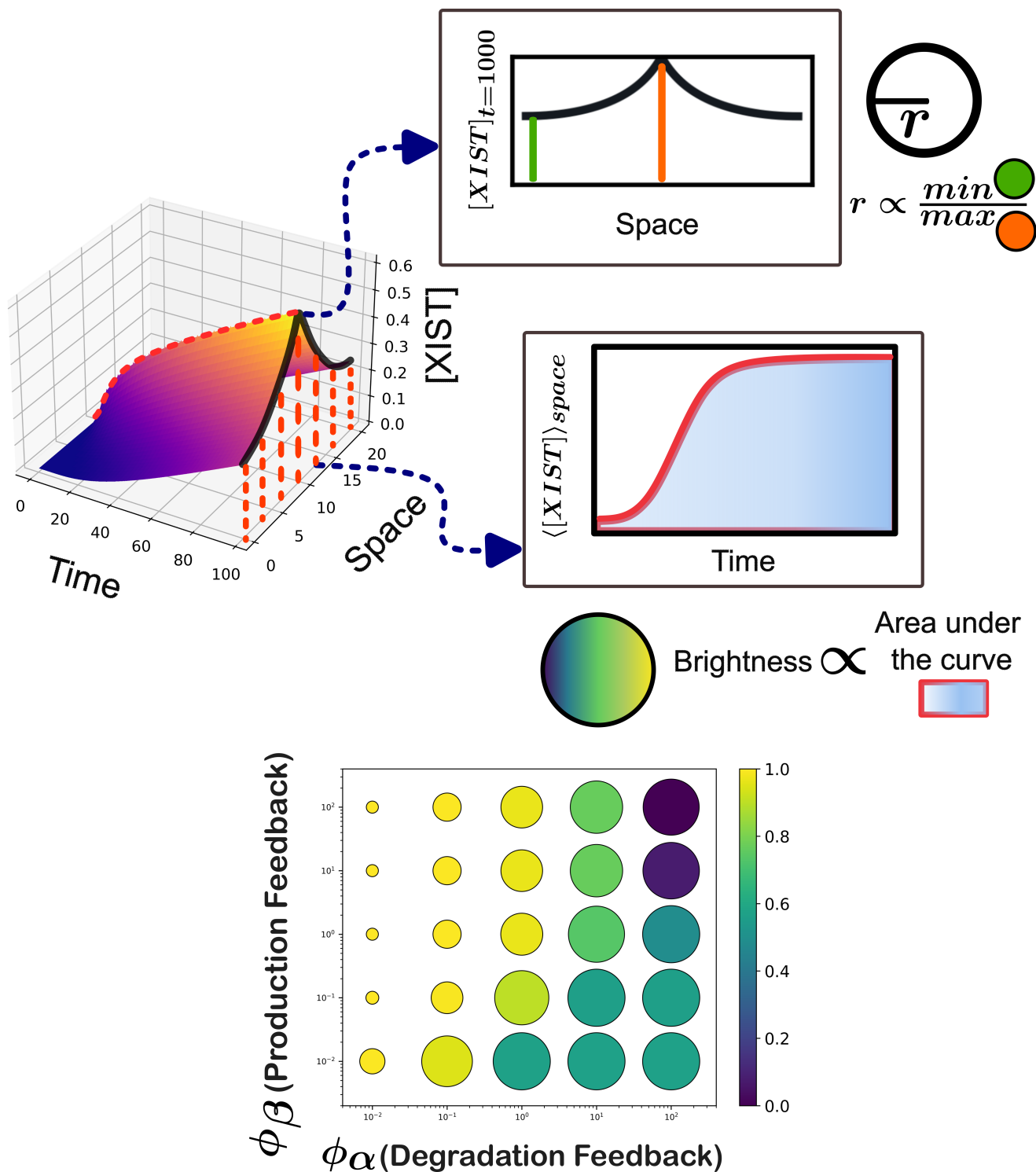


Fig. S3: The procedure for generating bubble plot from simulation data: Here each bubble represents the spatio temporal profile of XIST concentration. The larger bubbles represent more spatial spreading of XIST molecules and brighter bubbles represents rapid attainment of steady spatial profile. The parameters are same as used in Fig.S1.

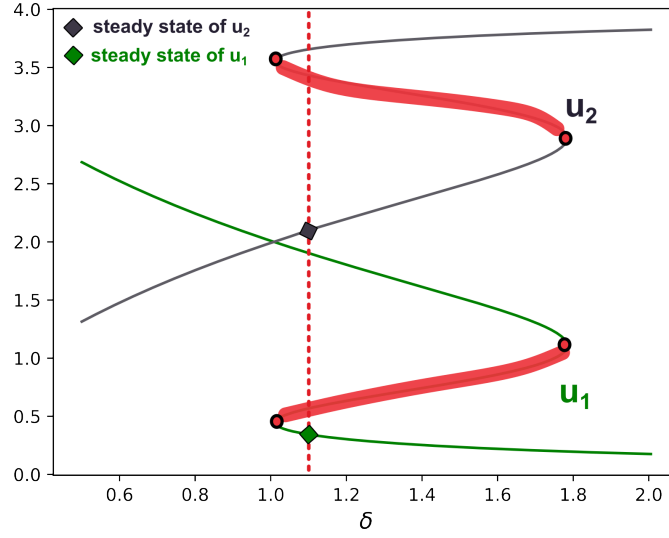


Fig. S4: Numerical continuation result of the XCI module. The parameters used are as follows: $\kappa_0 = 0.067$; $K = 1.0$; $\gamma = 1.0$; $n = 4$; $\alpha_{u_1} = \alpha_{u_2} = 0.05$; $\beta_{u_1} = \beta_{u_2} = 0.1$.

in Fig.S5. The parameter values used for the simulations are organized in the Table S1.

Fig.S5(A) represent the unpinned wave. To further establish the robustness of the unpinning we widened the space keeping other parameters fixed. The unpinned wave thus obtained is illustrated in Fig.S5(B) and the result implies that the unpinned state is truly unpinned arising out of the underlying dynamics of the system and it is independent of the length of simulated space.

Next we obtained the pinned wave from the unpinned wave in three ways and for each of them we tweaked only one of the parameters from the unpinned scenario. Fig.S5(C) represent the unpinned wave obtained by tweaking the feedback coefficient γ . Here we used $\gamma = 1$ for the region $2 < x < 18$ and $\gamma = 0.5$ otherwise. Thereafter, starting from the unpinned state of Fig.S5(A) we increased α_{u_1} and α_{u_2} from 0.05 to 0.06 keeping all other parameters fixed and that change in alpha value transformed the unpinned state into a pinned state and the pinned state is depicted in Fig.S5(D). Lastly, in Fig.S5(E) we illustrate the pinned state arising out of the increase in the inverse of feedback coefficient K from 1.0 to 1.2.

Next, we studied the way to control the extent of inactivation and for that purpose we chose a relatively less parameter K which modulates the feedback of the system. In Fig.S6 we portrayed the results for different K values. The Figs.S6 (A), (B), and (C) portray the spatio-temporal profile of X inactivation respectively for $K = 1.0$, $K = 1.1$ and $K = 1.2$. The results unravel that with increase in K , i.e., the inverse of the positive feedback coefficient, the extent of X chromosome inactivation decreases.

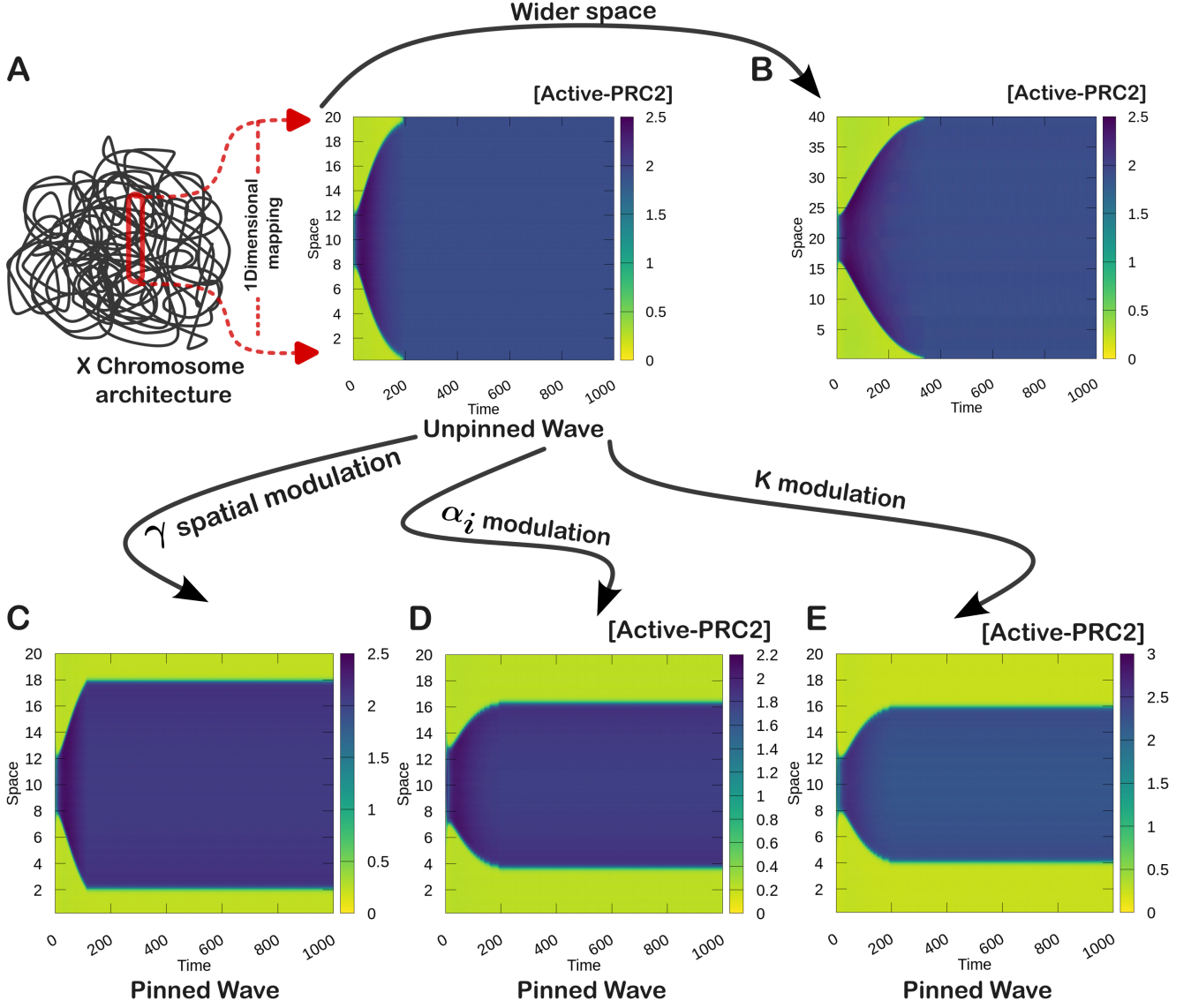


Fig. S5: Simulation result of XCI module for different parameter values: **(A)** Mapping of chromosomal structure in one dimension and the inactive wave propagation leading to unpinned wave for the parameters $u_1^0 = 0.3$; $u_2^0 = 2.0$; $\Delta x = 0.2$; $L = 20$; $\Delta t = 10^{-3}$; $D_{u_1} = 0.025$; $D_{u_2} = 10.0$; $\delta = 1.10$; $\kappa_0 = 0.067$; $K = 1.0$; $\gamma = 1.0$; $n = 4$; $\alpha_{u_1} = \alpha_{u_2} = 0.05$; $\beta_{u_1} = \beta_{u_2} = 0.1$; $n_{\text{stim}} = 1$; $t_1 = 2$; $t_2 = 4$. **(B)** The depiction of unpinned wave with parameters same as (A) but for a wider spatial region, i.e., $L = 40$. **(C)** Wave pinning achieved by spatial modulation of the parameter γ , other parameters are similar to that of (A). **(D)** Wave pinning attained by α_i modulation, here the parameters are same as (A) but for $\alpha_{u_1} = \alpha_{u_2} = 0.06$. **(E)** Wave pinning achieved by variation of K , parameters are same as (A) but for $K = 1.2$. (units arbitrary)

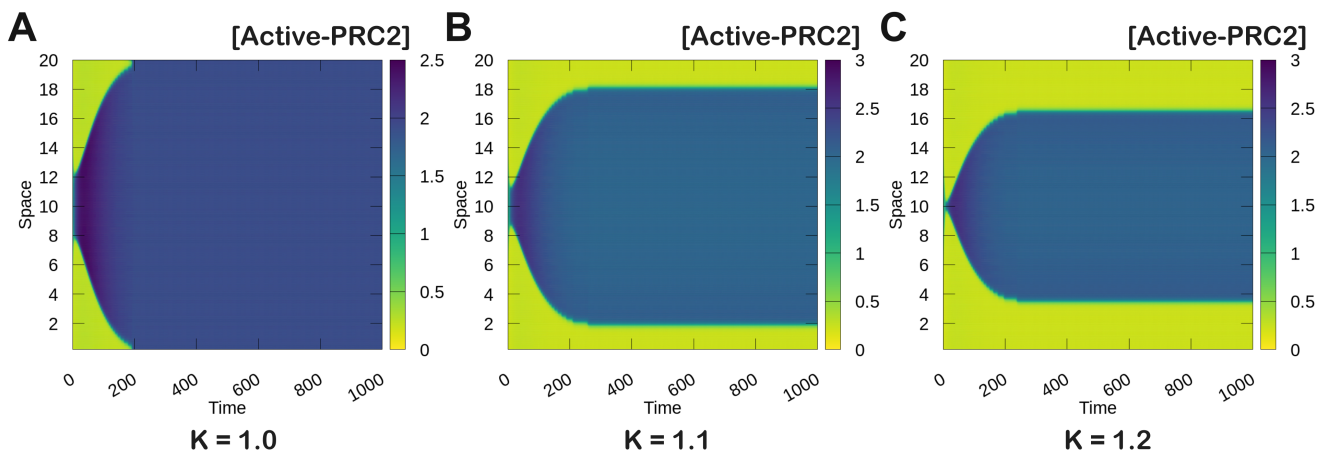


Fig. S6: Simulation result of XCI module for variation of the feedback coefficient K : (A) The parameter values are similar to Fig.S5(A), i.e., $K = 1.0$; (B) same as (A) but for $K = 1.1$; (C) same as (A) but for $K = 1.2$. (units arbitrary)

3.1.3 Effect of spatial constraint of chromatin on XCI

In the simulations carried out in previous subsections, we have assumed that the chromatin structure is continuously distributed throughout the one-dimensional space. Although this assumption is useful for exploring various dynamical features of the model, a more complete description requires considering the discrete nature of chromatin structure. In this subsection, we will examine the impact of the discrete nature of chromatin on the process of X chromosome inactivation.

In Figs.S8 and S9 we have portrayed different scenarios in terms of the discrete chromatin structures mapped in one spatial dimension. Here we have implemented the void space (white regions in our visualization) by distinctively assigning value of γ , i.e., γ effectively can have two values 1 and 0 depending on the spatial coordinate. From biochemical perspective this means the elimination of chromatin originated positive feedback at void spaces. The detailed description of the parameter values used for simulation are given in Table S2.

The results obtained for periodic and zero flux boundary condition reveals that the waves repeal each other and this repulsion is originated from the interplay between the active and inactive forms of PRC2. The detailed description of the spatio-temporal interaction that lead to the phenomenon of wave repulsion has already been discussed in the main text.

Moreover, the results also suggest that irrespective of the boundary conditions the steady spatial profile of the chromosomal activation is dependent on the final orientation of the chromatin structure. We have used this highly important feature of this model to justify our use of Xi chromosome

structure instead of Xa structure for 3D simulations.

The compaction depicted in Figs.S8(**G**) and S9(**G**) respectively for periodic and zero-flux boundary conditions has been carried out by defining the void space at $t = 0$ by setting $\gamma = 1.0$ for $0, 30 < x < 10, 40$ and $\gamma = 0.0$ otherwise. During the numerical simulation, we implemented the compaction by redefining the void space at each 1200^{th} time steps, i.e., at the $i * 1200^{th}$ time step ($i = \text{integer}$) of the simulation the void space is defined as $\gamma = 1.0$ for $0+i, 30-i < x < 10+i, 40-i$ and $\gamma = 0.0$ otherwise. The compaction stops when the terminal regions of the chromatin reaches a predefined point in space. Furthermore, to ensure the conservation of mass during this whole compaction process, we have mapped the concentration of u_1 and u_2 at each compaction step which we have termed as state mapping. The detailed procedure for state mapping during compaction is illustrated in Fig.S7.

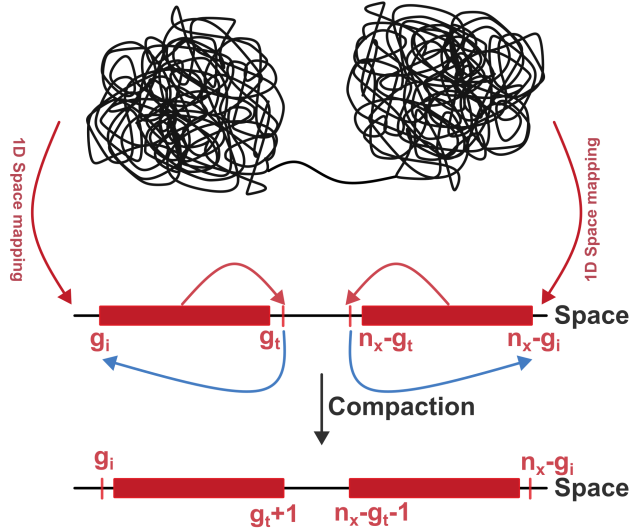


Fig. S7: The schematic diagram depicting details of state mapping during compaction.

The state mapping has been implemented as follows:

$$\begin{aligned}
 \text{mem} &= u_j(g_t + 1); u_j(g_i + 1 : g_t + 1) = u_j(g_i : g_t); u_j(g_i) = \text{mem} \\
 \text{mem} &= u_j(n_x - (g_t + 1)); \\
 u_j(n_x - (g_t + 1) : n_x - (g_i + 1)) &= u_j(n_x - g_t : n_x - g_i); u_j(n_x - g_i) = \text{mem}
 \end{aligned} \tag{S.11}$$

Where, g_i and g_t are indices defining the edges of the chromatin region at $t = 0$ and n_x is the total number of spatial grid points. mem is a dummy variable used during the process.

Periodic Boundary Condition

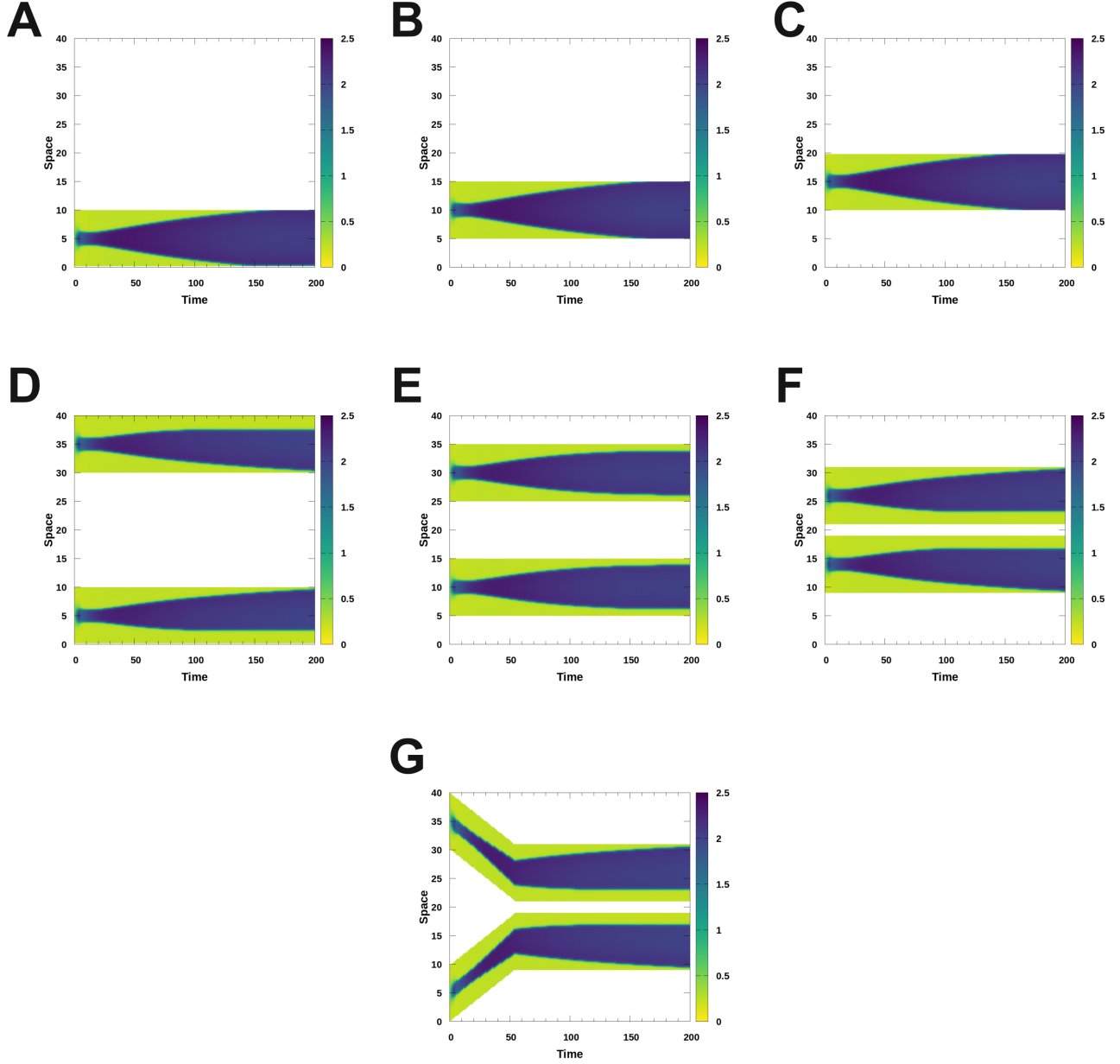


Fig. S8: Effect of spatial constraint on the process of XCI for **periodic boundary condition**. An itemized description of the parameters used and other specification of the simulations are provided in Table S2.

Zero-Flux Boundary Condition

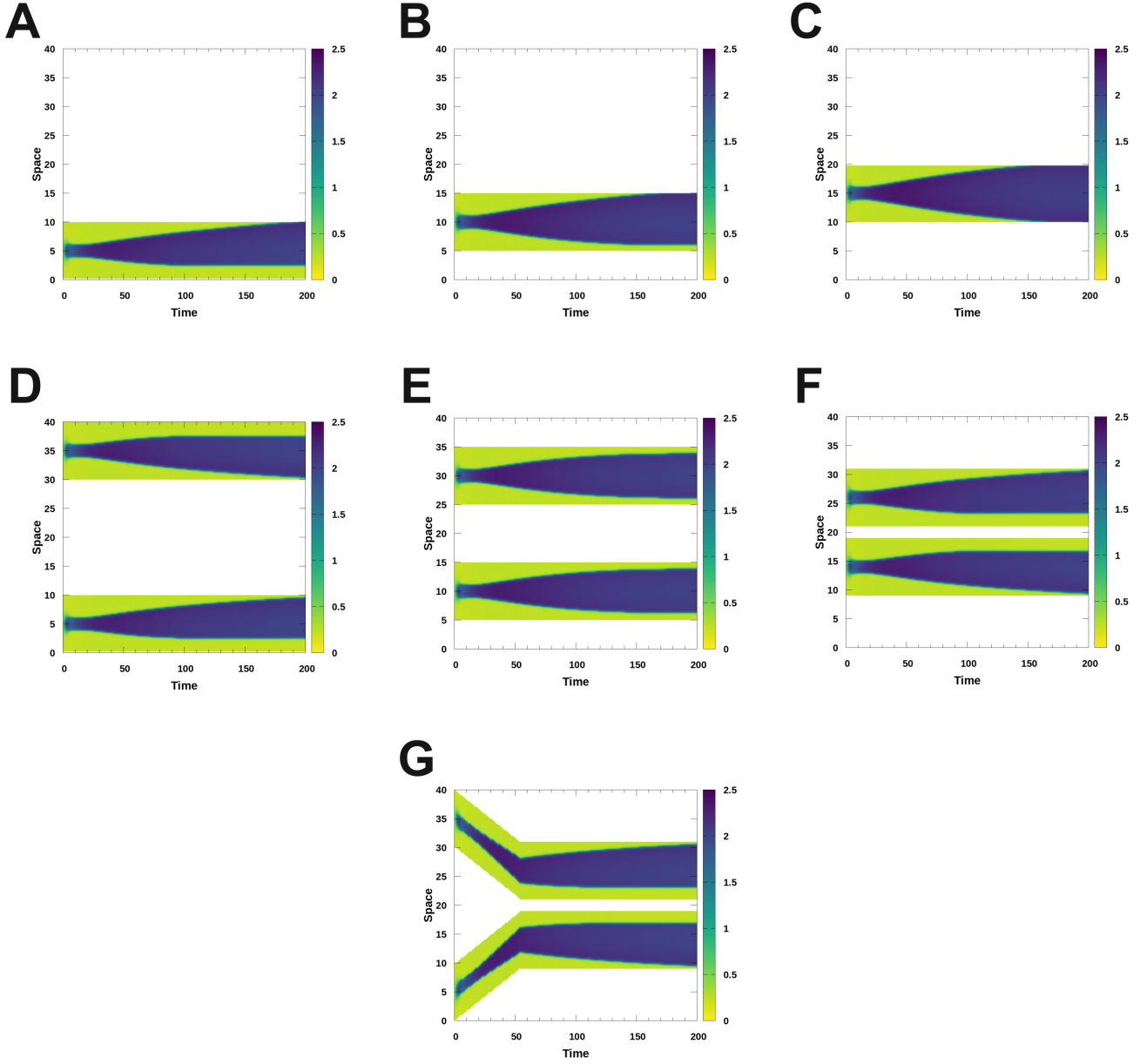


Fig. S9: Effect of spatial constraint on the process of XCI for **zero-flux boundary condition**. An itemized description of the parameters used and other specification of the simulations are provided in Table S2.

3.1.4 Integrating XIST module with XCI module through Tethering module

Subsequently, we integrated the XIST module and the X chromosome inactivation module by the tethering module. The governing principles of the tethering module have been provided in Sec.2.3. The simulation results are depicted and discussed in the main text. We have provided an elaborated account of the parameters used to get the results in Table S3.

3.1.5 A study of differential diffusivity of XIST molecules in chromatin region and void space

After the integration of the XIST module and XCI module, we inquired the effect of the differential diffusivity, i.e., region specific diffusivity of the XIST molecules. We have made two distinct regions, one where $\gamma = 1.0$ defining the chromatin region and another where $\gamma = 0.0$ defining the void space. To unveil the effect of differential diffusivity we have made D_X to adopt two values D_X^{chr} and D_X^{void} which represent diffusion coefficient of XIST molecules in the vicinity of chromatin and in the void space respectively. The simulation results thus performed is portrayed in Fig.S10, Fig.S11 and the utilized parameters is described in Table S5.

The results reveal that for tethering at an acceptable extent and speed to take place, diffusion coefficients of XIST molecules at both void space and chromatin should overcome a threshold value which will depend on the relative position of the chromatin in space with respect to the XIST transcription site. Once both of them reach the particular threshold value (i.e., 5 for D_X^{chr} and 7.5 for D_X^{void}) tethering extent and speed becomes directly proportional to either singly or jointly to the diffusion coefficients over void space and chromatin region.

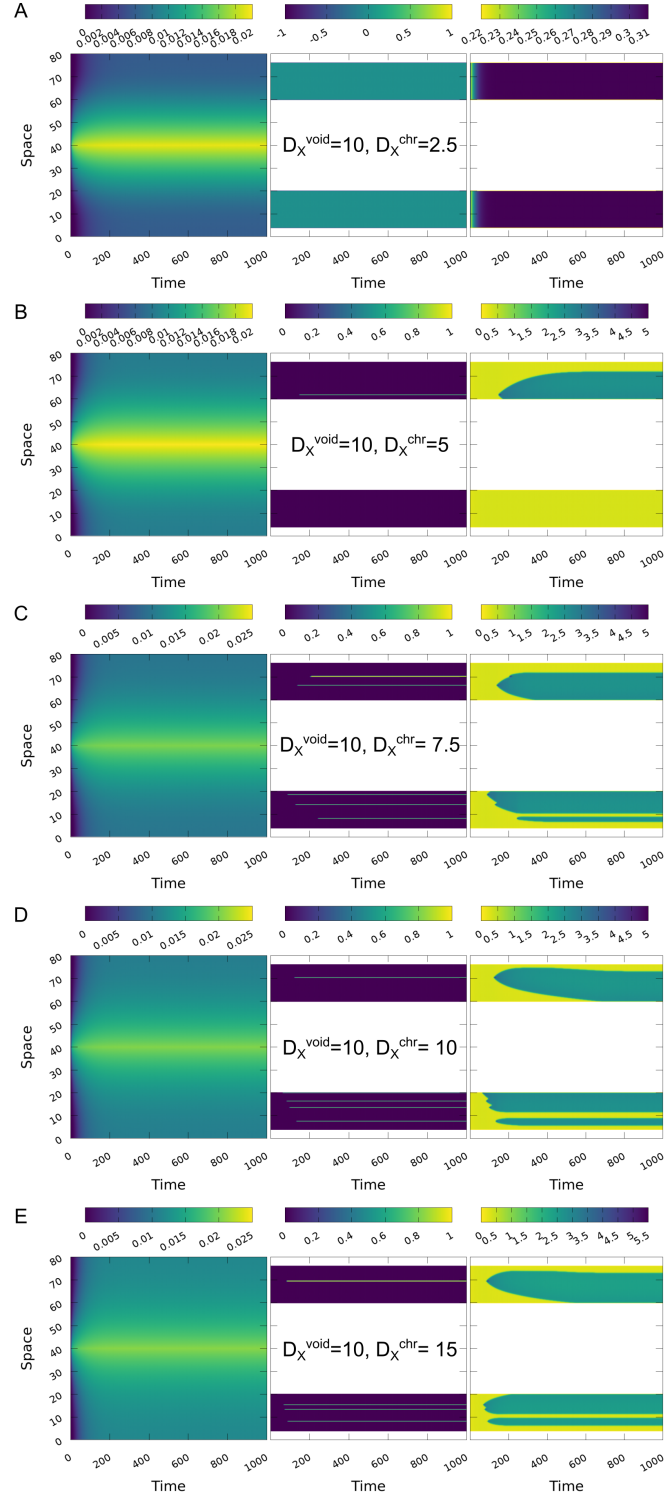


Fig. S10: Effect of region specific differential diffusivity of XIST molecules on the course of XIST tethering. The D_X^{void} has been fixed at 10.0 and D_X^{chr} has been varied among the values: **(A)** $D_X^{chr} = 2.5$; **(B)** $D_X^{chr} = 5.0$; **(C)** $D_X^{chr} = 7.5$; **(D)** $D_X^{chr} = 10.0$; **(E)** $D_X^{chr} = 15.0$.

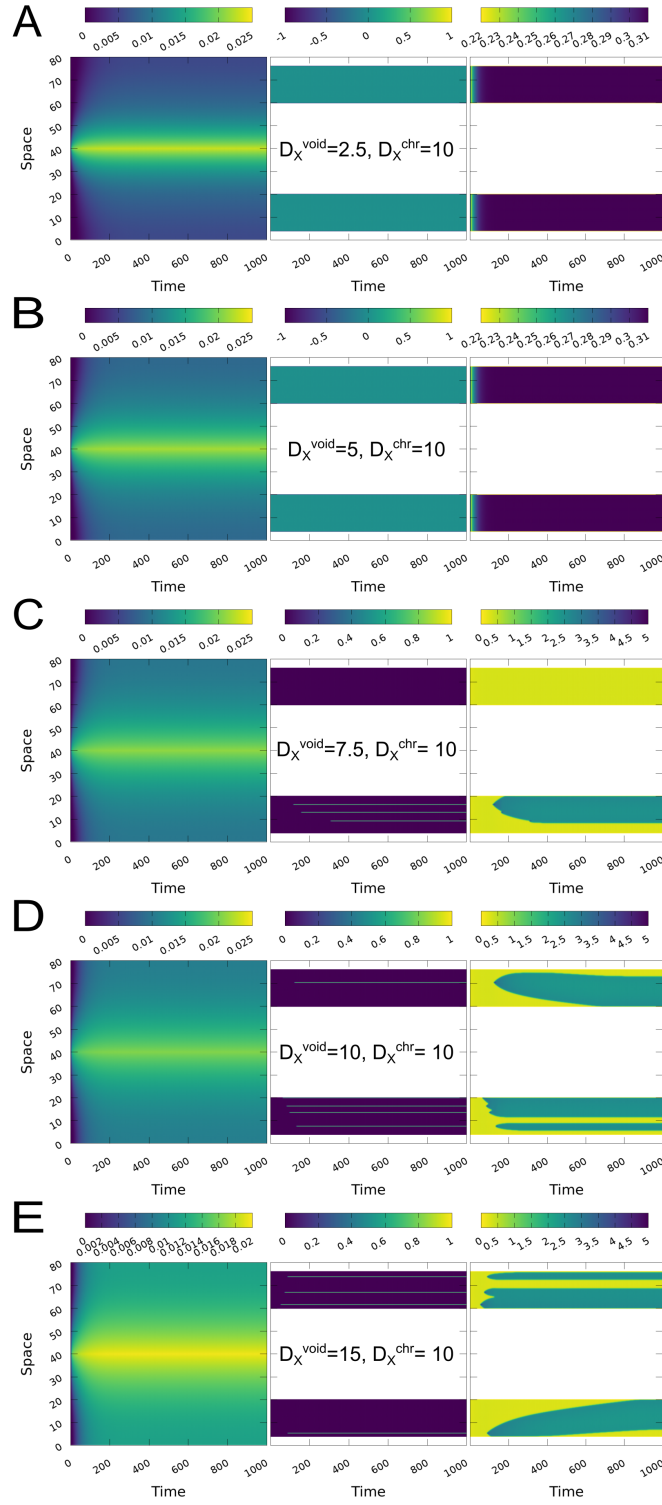


Fig. S11: Effect of region specific differential diffusivity of XIST molecules on the course of XIST tethering. The D_X^{chr} has been fixed at 10.0 and D_X^{void} has been varied among the values: (A) $D_X^{chr} = 2.5$; (B) $D_X^{chr} = 5.0$; (C) $D_X^{chr} = 7.5$; (D) $D_X^{chr} = 10.0$; (E) $D_X^{chr} = 15.0$.

3.1.6 Effect of temporal grid size Δt and spatial grid size Δx variation on the course of the simulation

To understand the effects of the temporal and spatial grid sizes used during the simulation on the overall outcome of X chromosome inactivation, we performed a systematic study in one dimension, as portrayed in Fig. S12. The study revealed that halving the temporal grid size Δt from 0.001 t.u. to 0.0005 t.u. has no effect on the simulation outcome in terms of the extent of inactivation, the time required to attain a steady state, and the spatial profile of wave pinning. However, halving the spatial grid size Δx from 0.2 s.u. to 0.1 s.u. has a profound effect on the extent of chromosomal inactivation. Conversely, the other two parameters—namely, the inactivation profile and the time required to attain a steady state—remain almost unchanged.

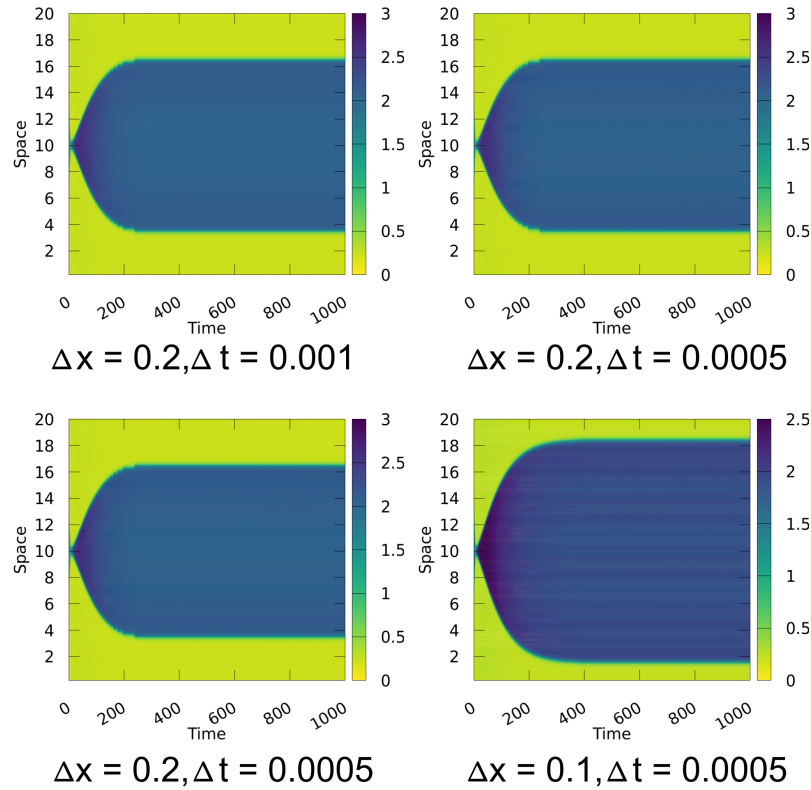


Fig. S12: Effect of temporal and spatial grid size variation on the chromosomal inactivation. The simulations were performed using the identical parameter values reported for Fig.S6(C) except the time and space grid size as shown in the figure.

3.2 Simulations performed in three dimensions

In the next stage of our study we explored the whole phenomenon of X chromosome inactivation using a reasonable three dimensional X chromosome architecture. Using the continuum model was the most significant bottleneck for simulating the model in three dimensions with discrete chromosome structure. The main challenge was to include the effect of chromosome structure into the spatio-temporal dynamics of X chromosome inactivation. We have overcome this obstacle by spatially modulating the feedback parameter γ , which is highly sensitive parameter towards the bi-stability of the system. As the bi-stability is the key tenet of the system to exhibit wave pinning, with diminished γ the system effectively gets converted to a mono-stable one. For the three dimensional model we have made γ space dependent, i.e., for each spatial point we calculated the lowest distance of that particular point from the chromosome architecture and then defined the effective γ for that point as follows:

$$\gamma(x, y, z) = \gamma_0 e^{-\kappa d} \quad (\text{S.12})$$

This suggests that both the feedback and the bi-stability of the system are influenced by the chromosome structure. For $d = 0$, where the spatial points reside on the chromosome structure, $\gamma = \gamma_0$. For very large d values, where the spatial points are far from the chromosome structure, $\gamma \approx 0$. This dependence of γ on the chromosome structure incorporates the essential influence of the chromosome's structure into the system's spatio-temporal landscape. Additionally, this approach to γ captures the transient movements of the chromosome in space and considers the structure as an averaged one. This is justified, as we have used the semi-empirical chromosome architecture derived from HiC data [8] and interpolated the chromosome structure to improve gene resolution. Moreover, the γ spatial modulation ensures that the γ on an average takes higher value for chromatin dense regions leading to bi-stability and chromosomal inactivation and vice versa. To facilitate reader convenience and clarity, we present the necessary details for the 3D simulation in an itemized format in the following text box.

3D simulation details

The entire procedure of 3D simulation is provided in a chronological order as follows:

1. We obtain the Cartesian co-ordinates (700 data points) of the chromosome structure from [8] and then we interpolate those 700 points to generate 20000 overall data points. In reality, the X chromosome has approximately 155 million base pairs and the increase in structural resolution of X chromosome later facilitated the comparison of simulated

result with reported experimental data by enhancing the gene resolution.

2. We then discretized the space according to the coordinates of the X chromosome structure and that resulted in a simulation volume in the shape of a parallelepiped. The details of the dimension in provided later in the Table S4 containing the parameter details.
3. Subsequently, we define the space dependent γ values according to the Eq.S.12 for XCI module and for the XIST module we fixed the X inactivation center, i.e., the point in 3D space with nonzero β_X value. Moreover, we have not implemented any chromatin structure dependency in the spatio-temporal dynamics of the XIST molecules as the production and spreading of the molecules occur at the very beginning of the X chromosome inactivation and the overall chromatin structure remains almost unchanged [9].
4. Thereafter, we utilized explicit Euler algorithm to simulate the PDEs described by Eqs.S.1, S.2 and S.3. We have implemented the tethering of XIST molecules following the conditions described in Eq.S.5. The detailed description of various parameters used are presented in Table S4. In this regard, we would like to mention that the Laplacian operators described in the equations S.1, S.2 and S.3 takes the three dimensional form which can be written as:

$$\nabla_{3D}^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \quad (\text{S.13})$$

And the corresponding central difference formula takes the following form:

$$\begin{aligned} \nabla_{3D}^2 u(x, y, z) = & \frac{u(x + \Delta x) + u(x - \Delta x) - 2u(x)}{\Delta x^2} + \frac{u(y + \Delta y) + u(y - \Delta y) - 2u(y)}{\Delta y^2} \\ & + \frac{u(z + \Delta z) + u(z - \Delta z) - 2u(z)}{\Delta z^2} \end{aligned} \quad (\text{S.14})$$

5. Next, we map the x-linked genes of a particular species (mouse, in the context of this paper) to the previously mentioned polymeric structure with 20000 segments and mark them as on or off according to the experimental results [10]. Lets take a test case to explain the procedure: The length of the mouse genome is ≈ 169 Mbp [11] which

implies that each segment of the used polymeric structure correspond to ≈ 8473 bp. The XIST gene for mouse is located at the region 102503979 to 102526839 [11] and mapping of the XIST gene to the polymeric structure reveals that the XIST gene is located in between 12097 th and 12099 th segment of the same. Similarly, we have mapped all the genes that are known to escape the process of inactivation and tried to capture as many of them as possible through the simulation.

6. For the basal 3D simulation we have chosen the tethering sites randomly with the exception of X Inactivation Center. The experimental findings have revealed that X inactivation center is located in such a way that there is very few number of silenced genes in its' vicinity [12]. To include this effect, during the simulation we have not allowed the tethering of XIST molecules inside the sphere of radius 30 spatial units, centered around the XIC (12098 th segment of the polymeric chain), as this restriction ensures the XIST tethering to the peripheral region which is less dense.
7. Finally, we analyzed the simulation results and compared them with the experimental observations.

To demonstrate the effect of γ variation on the bi-stability as well as the wave pinning phenomenon we have studied the wave-pinning in one dimension using the parameter values that were used for the three dimensional simulation. The study illustrated in Fig.S13(C) reveals that for the our current model the maximum distance from the chromatin structure at which the bi-stability as well as wave pinning exists is $d_c^{1D} = 0.022$ spatial units. Moreover, we have also noticed that the inclusion of diffusion in the picture makes bi-stability highly sensitive to the variation of the parameter γ . The numerical continuation result depicted in Fig. S13(A) indicates the sustenance of bi-stability in zero dimensional ODE system even when there is 50% decrease in the value of γ . On the other hand, the panel (C) of the same figure reveals destruction of bi-stability in the one dimensional spatially extended system even at the 1.143% decrease in the value of γ . Hence, we can safely infer that for the three dimensional system γ sensitivity will be even higher and $d_c^{3D} < 0.022$.

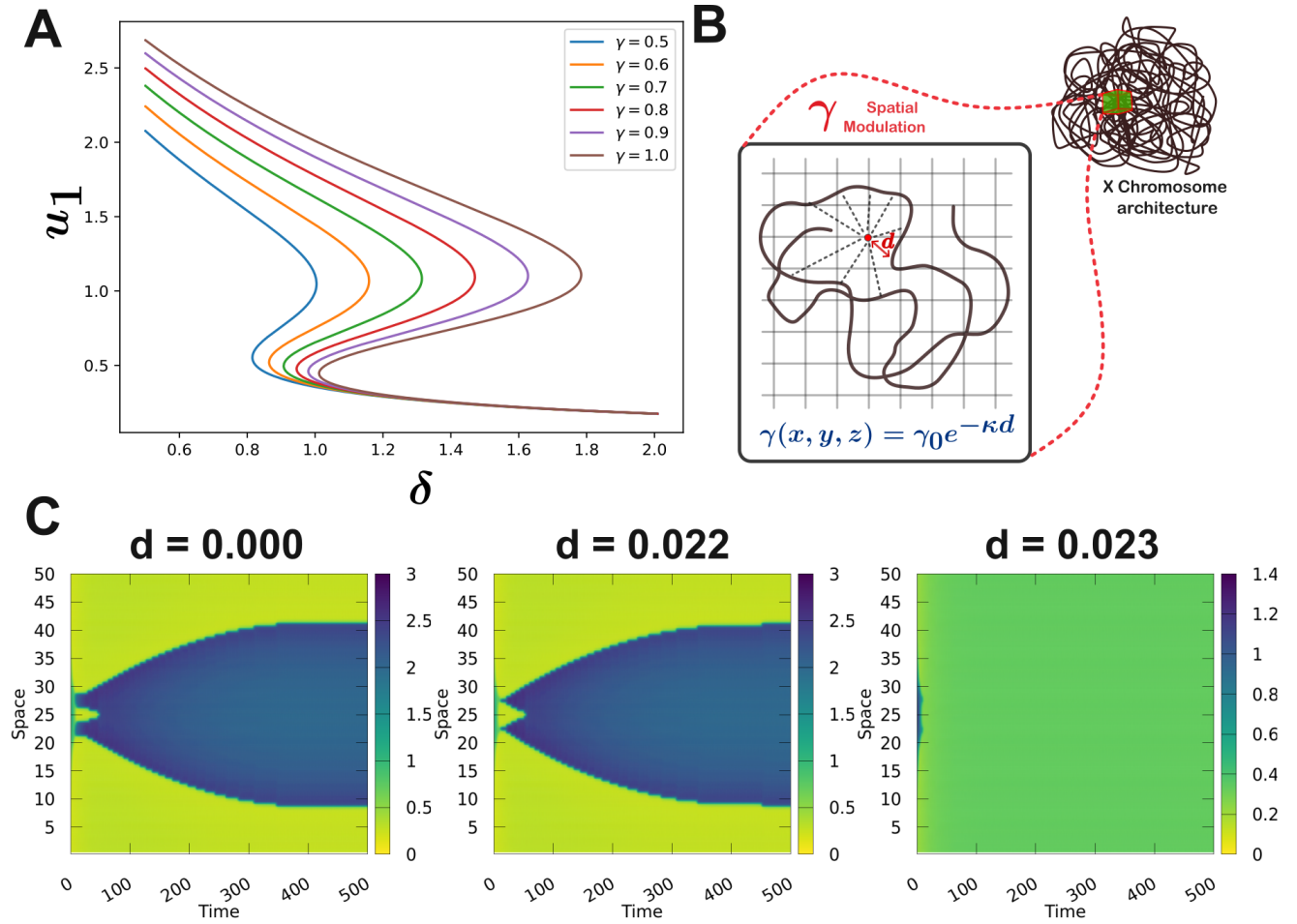


Fig. S13: **(A)** Depiction of variability of the bi-stability with variation in γ . The result implies high sensitivity of the parameter γ on bi-stability. **(B)** A schematic diagram roughly illustrating the method of the spatial modulation of γ . **(C)** Elimination of bi-stability and hence wave-pinning phenomena with increased distance from the chromatin structure.

Insights on length and time scale of the 3D simulation

The typical length scale of the X chromosome in mammalian cells is approximately $1 \mu\text{m}$ [13]. In our 3D simulation framework, the average length of the simulated box is approximately 70 spatial units, meaning one spatial unit corresponds to $0.014 \mu\text{m}$ of physical length. Since XIST is the key driver of X chromosome inactivation dynamics, we normalized the time with one thousandth of the median half-life of XIST RNA molecules, which is approximately 0.004 hours or 14.4 seconds [14]. This normalization implies that one unit of the diffusion coefficient in our simulation corresponds to approximately $1.36 \times 10^{-5} \mu\text{m}^2/\text{sec}$ in physical

units. For numerical reasons, we used a diffusion coefficient of XIST molecules $D_X = 25$, which corresponds to approximately $3.4 \times 10^{-4} \mu\text{m}^2/\text{sec}$. The sub-nuclear diffusion coefficient of mRNA molecules ranges from 5×10^{-3} to greater than $1 \mu\text{m}^2/\text{sec}$ [15]. Considering that lncRNA are much heavier than mRNA molecules, the diffusion coefficient of XIST used in our simulation falls within a reasonable range, being less than the lower bound of the diffusion coefficient of mRNA molecules.

3.3 Analysis of 3D simulation data and comparison with experimental results

Once we obtained the simulation the new reasonable step was to analyze the data and compare the result with experimental results. We have used the gene expression data reported in Ref.[10] and mapped the genes in our chromosomal structure. We have considered a gene as escapee if its' expression probability is greater than 0.1. The result thus obtained is portrayed in Figure 5. Once we have the experimental results mapped to the chromosomal structure that we have used, we compare the simulation results with experimental results. We have categorized a gene as active according to the simulation, if there is at least 50 percent agreement between the simulation result and the experimental result. A detailed illustration of the condition is provided in Fig.S14. The analysis of the results are provided in the main text.

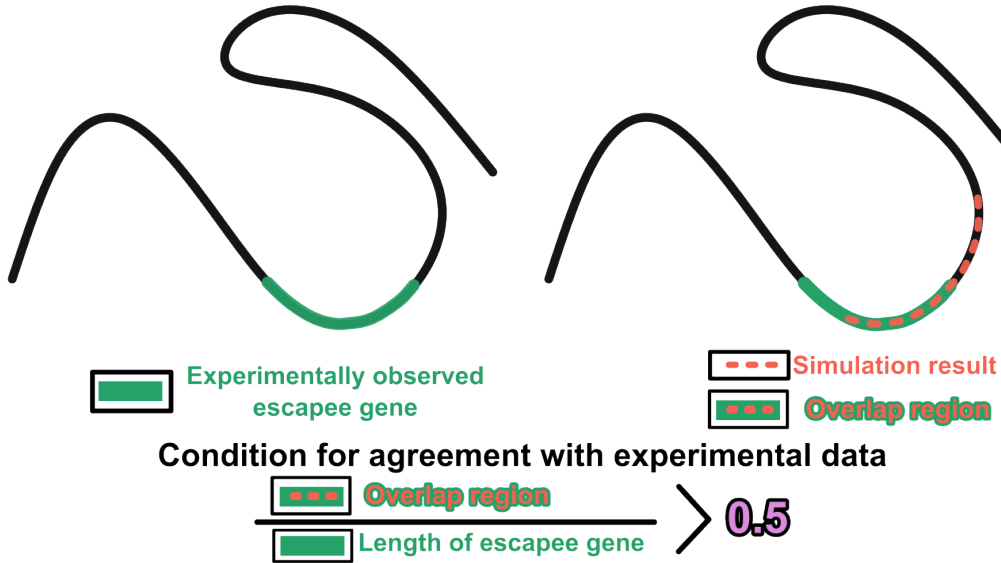


Fig. S14: Schematic representation of the condition for agreement between simulated and experimental results.

3.4 Implementation of Imposed Tethering

3.4.1 One Dimensional Modeling

Finally, we asked what the system dynamics would look like if the tethering sites for XIST molecules were predetermined. To address this, we developed a prototype one-dimensional model in which tethering is permitted only at spatial locations that are integer multiples of 20 units. The results (Fig.S15) show that although the tethering positions are fixed and independent of the XIST module, the timing of site activation remains governed by the XIST component of the model. The parameters used for the simulation are described in Table S6.

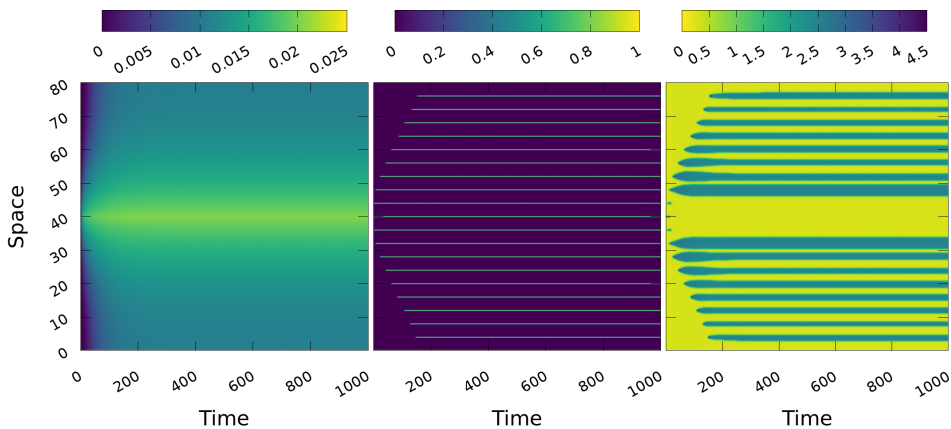


Fig. S15: The progression of XCI is examined under the condition that XIST molecules can bind only at spatial locations corresponding to integer multiples of 20 units. The same units are used as in Figs. S11 and S10, except that for the entire spatial domain we set $\gamma = 1.0$ and $D_X = 10.0$.

3.4.2 Three Dimensional Modeling

As a natural extension of our one-dimensional analysis, we implemented the same simulation framework in our three-dimensional model. In this setup, we incorporated the XIST tethering sites reported in the experimental study by Engreitz et al. [12], conducted in female mouse embryonic stem cells. We extracted the early XIST enrichment sites reported in Figure 3(F) of the Engreitz et al. paper. We converted these experimental locations to the corresponding segments on our 20,000-unit polymeric chain, resulting in a total of 44 tethering sites. Finally, we performed the 3D simulation, allowing XIST molecules to bind to these designated sites. Subsequently, we analyzed the simulation data to identify escapee genes captured under the imposed tethering conditions. The results indicate that, under the same criteria for agreement with experimental observations discussed earlier, the model successfully reproduced only 8 of the 38 known escapee genes. The

spatial distribution of these captured genes is illustrated in Fig. S16. We used the same set of parameters as in our previous three-dimensional simulation, as listed in Table S4.

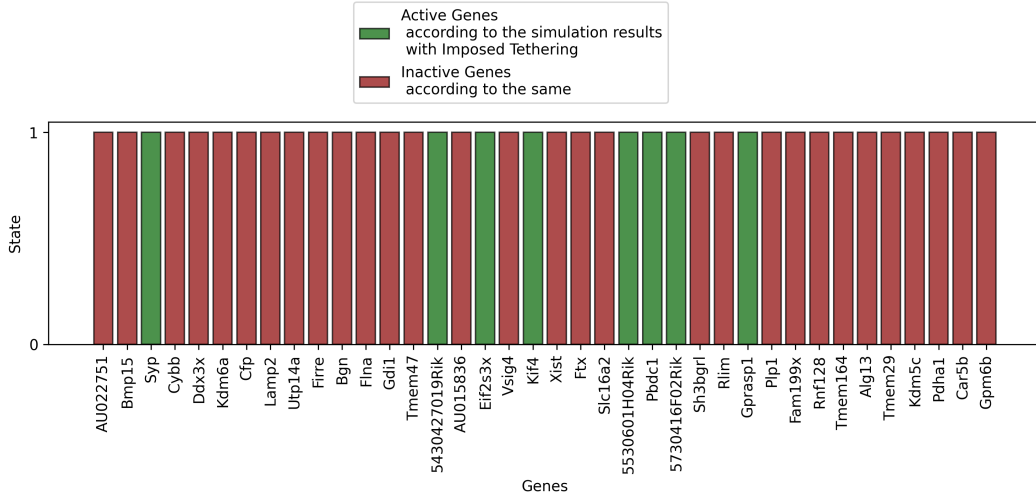


Fig. S16: Distribution of simulated escapee genes with imposed tethering sites. The escapee genes which are captured in simulation are depicted by green colored bars and those genes which are not captured in simulation are red colored.

3.4.3 Comparison of imposed tethering with random tethering

To select a representative model and to assess the general behavior of the three-dimensional model, we performed 20 independent three-dimensional simulation runs with different sets of random XIST tethering sites on the X chromosome. In these simulations, tethering was implemented in a purely random manner, excluding a spherical region of radius 30 spatial units centered at the X-inactivation center (XIC). The resulting data were analyzed with metrics described below and were compared with results from an "imposed tethering" simulation informed by experimental data. For the imposed tethering condition, potential XIST tethering sites were identified from the experimental study by Engreitz et al. (2013) [12] and subsequently mapped to the polymer model according to its length, yielding 44 tethering sites in total. Since these experimentally derived sites were largely positioned away from the XIC, no spatial exclusion constraint was applied, unlike in the random tethering simulations.

To evaluate the model runs, we used a set of 38 experimentally observed escape genes (denoted as true positives, or TP), and a set of 430 silenced genes (true negatives, or TN), i.e. mouse X genes that were not observed as escapees[10]. Incorrectly predicted escape genes are false positives (FPs), and incorrectly predicted silenced genes are false negatives (FNs). We used sensitivity, defined as

TP/(TP+FN), specificity, defined as $TN/(TN+FP)$, false positive rate, defined as $FP/(FP+TN)$, precision, defined as $TP/(TP+FP)$, and accuracy, defined as $(TP+TN)/(TP+TN+FP+FN)$ to evaluate the model runs. We summarized the performance of the 20 runs in Table S7. Overall, the prediction of escape genes is significantly associated with the observed ones ($p < 10^{-5}$, Fisher's exact test. Odds ratio 2.14. Table S8)

We defined a gene to be 'silenced' if more than 50% of its corresponding genomic coordinates are at the inactive state at the final time point, and it is an escape gene otherwise. To show that the main conclusions of our manuscript are not overly sensitive to the choice of this threshold, we used two alternative thresholds, 20% and 80% to test our model performance. We found that the stable escape regions appeared regardless of the threshold. In addition, we used an alternative assumption that if the +1 position of the gene is repressed at the last time point, then the gene is considered silenced. The simulation results from this assumption are very similar to those from the threshold-based assumption S17.

One representative random-tethering simulation (green dots in Fig.S17) was selected ($p < 10^{-5}$, Fisher's exact test. Odds ratio 2.85), and its results were presented in Figure 5 of the main text. Across 20 runs, random tethering runs showed a mean sensitivity of 0.3342 (median: 0.3026) and a mean specificity of 0.8102 (median: 0.8255). We found that the imposed tethering (purple dots in Fig.S17) represents a special case of random tethering. Importantly, the XIST gene was active in all random tethering simulations, but was inactive in the imposed tethering simulation.

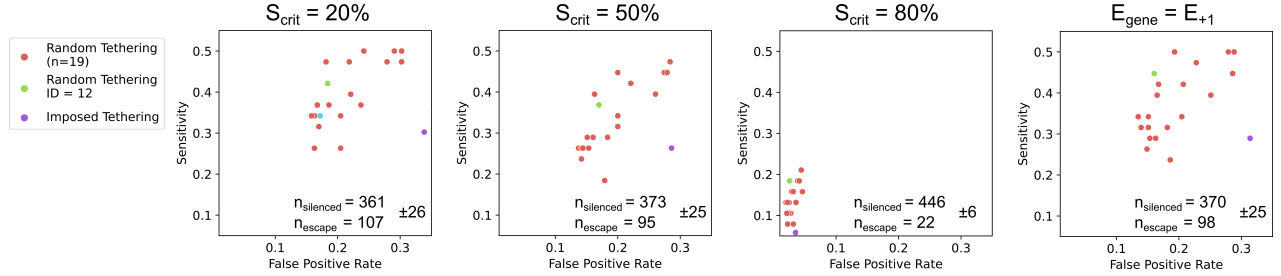


Fig. S17: Model performance with the main and alternative assumptions for determining silenced and escaped genes. Sensitivity and false positive rate are shown for 20 model realizations with random XIST tethering sites, 1 model with imposed tethering sites inferred from experiment, and 1 model with dynamic chromosome structure. A parameter S_{crit} is varied to test the robustness of the conclusions for the first 3 conditions. S_{crit} determines the percentage of repressed genomic coordinates of a gene at the last time point of a simulation, above which the gene is considered silenced. In the fourth assumption, a gene is silenced if the +1 position of the gene is repressed at the last time point and is otherwise an escaped gene. The mean numbers of silenced genes and escaped genes and the standard deviations of the numbers across 20 model realizations are indicated in the plots.

3.5 Mixed pinning

We have demonstrated the coexistence of intrinsic pinning and forced pinning (implemented via spatial modulation) in our 1D model. The results, shown in Fig S18, illustrate this combined effect. In the middle panel, the darker regions ($x < 10$, $x > 70$, and $30 < x < 50$) indicate where the spatial modulation was applied for forced pinning. The rightmost panel shows the actual pinning scenario, which manifests both the forced pinning in the modulated regions and the intrinsic pinning arising from the system's dynamical nature. Same parameter values as in Fig.S14 (Table S6) have been used for this particular simulation except the spatially dependent γ values, i.e., $\gamma = 0$ for $x < 10$, $x > 70$, and $30 < x < 50$ and $\gamma = 1$ otherwise.

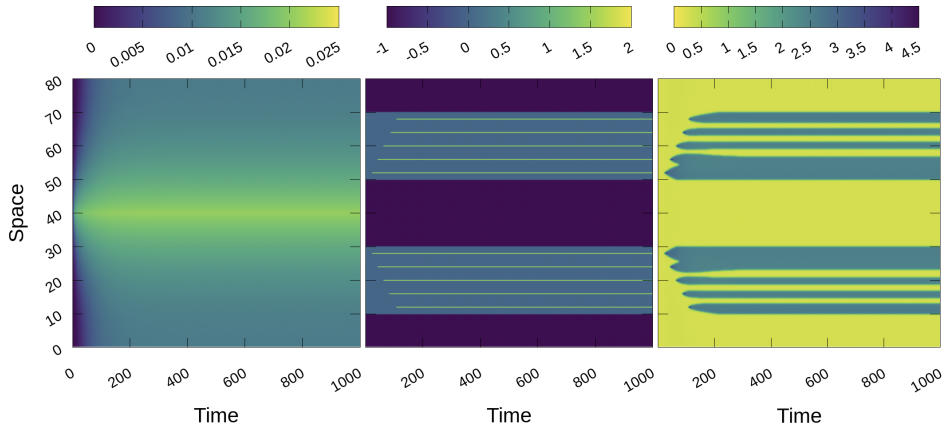


Fig. S18: Coexistence of intrinsic pinning and forced pinning during XCI.

3.6 Radial distribution of escaped and silenced regions during XCI

To understand the spatial organization and relative distribution of the escaped and silenced regions during X-chromosome inactivation (XCI), we analyzed their radial positioning. Specifically, we computed and compared the radial distribution profiles of these regions for both experimental data and simulation results. As a first step, the radial distance of each of the 20,000 polymeric segments representing the chromosome was determined using the following relation:

$$\tilde{r}_i = \sqrt{(x_i - x_c)^2 + (y_i - y_c)^2 + (z_i - z_c)^2} \quad (\text{S.15})$$

where $\tilde{r}_i$ is the radial distance and x_i, y_i, z_i denote the Cartesian coordinates of the i^{th} segment in the polymeric chain, x_c, y_c, z_c represent the central reference coordinates used for evaluating the radial distance. The central reference was defined as $q_c = (q_i^{\max} + q_i^{\min})/2$, with $q = x, y, z$. This approach ensures that the computed distances are measured consistently relative to the geometric center of

the spatial configuration. Using these radial distances, we further constructed Kernel Density Estimation (KDE) plots to visualize the radial distribution patterns for both the experimental and simulated datasets. These KDE plots provide an intuitive, continuous representation of the relative positioning of the escaped and silenced regions, facilitating a direct comparison between experimental observations and model predictions. The resultant distribution profiles, illustrating this comparison, are shown in Fig. S19.

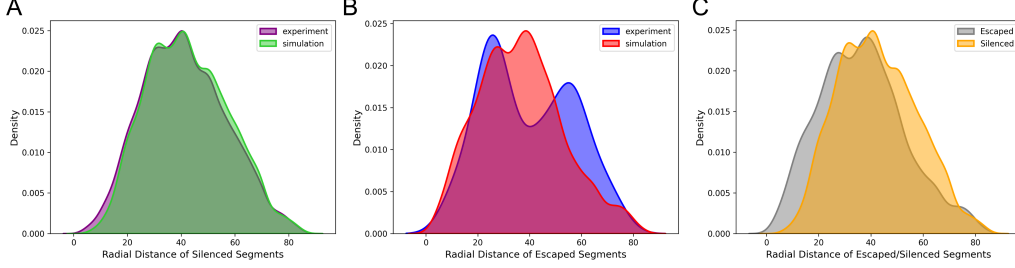


Fig. S19: Kernel Density Plots for the radial distance of the (A) silenced regions in experiment and in simulation; (B) escaped regions in experiment and in simulation; (C) escaped and silenced regions in simulation.

3.7 3D Simulation with dynamic chromosome structure

As an extension of our study, we performed a three-dimensional simulation of the dynamic chromatin structure using parameter values consistent with the representative run discussed previously. In this simulation, we implemented a dynamic transition where, starting from an active X chromosome (Xa) configuration, the coordinates are updated every $n_{move} = 5,000$ time steps. The system reaches the final inactive X chromosome (Xi) configuration at the $n_{time} = 500,000$ time step.

The structural configurations for both the active and inactive X chromosomes were obtained from Lappala et al. [8]. To prevent artifacts arising from translational motion, we ensured a fixed center of mass for both the initial and final structures. The equations governing the structural dynamics of the X chromosome are given by:

$$q_i(t + n_{move}\Delta t) = q_i(t) + \left[\frac{q_i^{in} - q_i^{ac}}{n_{time}} n_{move} \right] \quad (\text{S.16})$$

where $q_i = \{x, y, z\}$ represents the coordinates of the i^{th} segment of the polymeric chain. For a chain length of 20,000 units, the index i ranges from 1 to 20,000. The boundary conditions for the

simulation are: at $t = 0$: $q_i(0) = q_i^{ac}$ (Active X chromosome) and at $t = n_{time}\Delta t$: $q_i(n_{time}\Delta t) = q_i^{in}$ (Inactive X chromosome). The simulation results are illustrated in Fig.S20 and Movie S1.

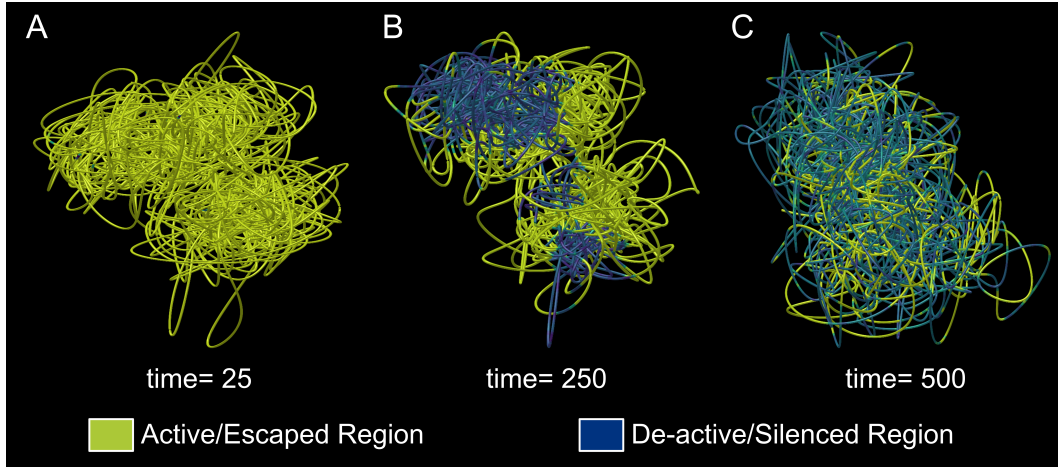


Fig. S20: Three-dimensional simulation results using a dynamic chromosome structure. Images were taken for three different time points during the simulation: **(A)** Time= 25 time units; **(B)** Time= 250 time units; **(C)** Time= 500 time units. Active X chromosome structure (Xa) inferred by Lappala et al. (Lappala et al. 2021) was used as the initial configuration and the inferred inactive X chromosome structure (Xi) was used as the final configuration. Interpolation was performed for each genomic coordinate to obtain a smooth transition between the two states. XIST tethering sites were the same as the best-performing simulation with random tethering sites (Run 12). Images were taken from the same azimuthal angle.

The performance of the simulation with dynamic chromosome structure was compared with all other simulation runs using the metrics described earlier (Fig.S21). The simulation had an overall accuracy close to the best (80.98%) among all models, but it only captured 9 out of 38 escape genes (the representative static structure simulation captured 14 escapee genes). The model achieved high specificity (86.05%), thanks to larger-than-average inactive chromosome regions, with an expense of a low sensitivity (23.68%, below 25 percentiles of the random tethering models with fixed structures). Therefore, the model's performance is comparable, but not superior, to that of other model configurations.

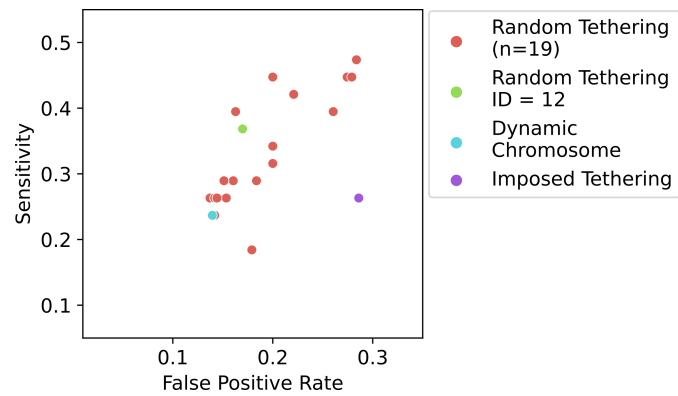


Fig. S21: Performance of the simulation with dynamic chromosome structure compared with other simulation runs. Sensitivity and false positive rate of models with random tethering sites, the model with imposed tethering sites inferred from experiment and the model with dynamic chromosome structure are shown.

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List of Supplementary Tables

Supporting Information 1 $\Rightarrow$ Table S1

Title : Parameters used to obtain results portrayed in Figure S5

Supporting Information 2 $\Rightarrow$ Table S2

Title : Parameters used to obtain results portrayed in Figures S8 and S9

Supporting Information 3 $\Rightarrow$ Table S3

Title : Parameters used to obtain results of integrated module (Figure 4 in main text)

Supporting Information 4 $\Rightarrow$ Table S4

Title : Parameters used to obtain results of 3D module (Figure 5 in main text)

Supporting Information 5 $\Rightarrow$ Table S5

Title : Parameters used to obtain results of differential diffusivity of the XIST molecules in one spatial dimension (Figures S10 and S11 in Supplementary File)

Supporting Information 6 $\Rightarrow$ Table S6

Title : Parameters used to implement imposed tethering in one dimension (Depicted in Figure S14 of the Supplementary File)

Supporting Information 7 $\Rightarrow$ Table S7

Title : Performance statistics for 20 model realizations with random XIST tethering sites.

Supporting Information 8 $\Rightarrow$ Table S8

Title : Confusion matrix aggregated across 20 model realizations with random XIST tethering sites.

Supporting Information 9 $\Rightarrow$ Table S9

Title : Compiled table of parameters used in different modules with brief justification.

4 Supplementary Tables

Table S1: Parameters used to obtain results portrayed in Fig. S5

Figure	Parameters
Fig. S5 (A)	$u_1^0 = 0.3; u_2^0 = 2.0; \Delta x = 0.2; L = 20; \Delta t = 10^{-3};$ $D_{u_1} = 0.025; D_{u_2} = 10.0; \delta = 1.10; \kappa_0 = 0.067; K = 1.0; \gamma = 1.0; n = 4;$ $\alpha_{u_1} = \alpha_{u_2} = 0.05; \beta_{u_1} = \beta_{u_2} = 0.1; n_{\text{stim}} = 1; t_1 = 2; t_2 = 4$
Fig. S5 (B)	Same as (A) but for $L = 40$
Fig. S5 (C)	Same as (A) but for $\gamma = 1$ at $(2 < x < 18)$ and $\gamma = 0$ otherwise
Fig. S5 (D)	Same as (A) but for $\alpha_{u_1} = \alpha_{u_2} = 0.06$
Fig. S5 (E)	Same as (A) but for $K = 1.2$

Table S2: Parameters used to obtain results portrayed in Fig. S8 and Fig. S9

Figure	Parameters (units arbitrary)
Fig.S8 (A)	$u_1^0 = 0.2683$; $u_2^0 = 2.0$; $\Delta x = 0.2$; $L = 40$; $\Delta t = 10^{-3}$; $D_{u_1} = 0.025$; $D_{u_2} = 10.0$; $\delta = 1.20$; $\kappa_0 = 0.067$; $K = 1.1$; $n = 4$; $\alpha_{u_1} = \alpha_{u_2} = 0.05$; $\beta_{u_1} = \beta_{u_2} = 0.1$; $n_{\text{stim}} = 1$; $t_1 = 2$; $t_2 = 4$ $\gamma = 1.0$ (for, $0 < x < 10$) and $= 0.0$ otherwise; Boundary= Periodic
Fig.S8 (B)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $5 < x < 15$) and $= 0.0$ otherwise
Fig.S8 (C)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $10 < x < 20$) and $= 0.0$ otherwise
Fig.S8 (D)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $0 < x < 10$, $30 < x < 40$) and $= 0.0$ otherwise
Fig.S8 (E)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $5 < x < 15$, $25 < x < 35$) and $= 0.0$ otherwise
Fig.S8 (F)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $9 < x < 19$, $21 < x < 31$) and $= 0.0$ otherwise
Fig.S8 (G)	Same as Fig.S8(A) but for: $\gamma = 1.0$ (for, $0 < x < 10$, $30 < x < 40$) and $= 0.0$ otherwise initially, The chromatin regions move one grid every 1200th time step till: $\gamma = 1.0$ (for, $9 < x < 19$, $21 < x < 31$) and $= 0.0$ otherwise
Fig.S9 (A)-(G)	Same as Fig.S8 (A)-(G) but for: Boundary= Zero-Flux

Table S3: Parameters used to obtain results of integrated module (Figure 4 in main text)

Modules	Parameters (units arbitrary)
Simulation	$\Delta x = 0.2; \Delta t = 10^{-3}; L = 80; \text{Boundary} = \text{Zero-Flux}$
XIST	$[XIST]_0 = 0; n_\beta = 4; n_\alpha = 4; D_X = 1.0; \alpha_X = 0.01;$ $\beta_X = 1.0 \text{ (for } x = 40 \text{) and } = 0.0 \text{ (otherwise);}$ $\phi_\beta = 0.1; \phi_\alpha = 1.0;$
XCI	$u_1^0 = 0.3; u_2^0 = 2.0; D_{u_1} = 0.025; D_{u_2} = 10.0; \delta = 1.10;$ $\kappa_0 = 0.067; K = 1.2; \gamma = 1.0; n = 4; \alpha_{u_1} = \alpha_{u_2} = 0.05;$ $\beta_{u_1} = \beta_{u_2} = 0.1$
Tethering	$n_{\text{thr}} = 10; n_{\text{total}} = 400; \phi_{\text{thr}} = 0.5$

Table S4: Parameters used to obtain results of 3D module (Figure 5 in main text)

Modules	Parameters (units arbitrary)
Simulation	$\Delta x = \Delta y = \Delta z = 0.5$; $\Delta t = 10^{-3}$; $L_x = 70$; $L_y = 72.5$; $L_z = 70.5$; Boundary= Zero-Flux
XIST	$[XIST]_0 = 0$; $n_\beta = 4$; $n_\alpha = 4$; $D_X = 25.0$; $\alpha_X = 0.01$; XIC= 12098; $\beta_X = 10.0$ (for XIC) and = 0.0 (otherwise); $\phi_\beta = 0.01$; $\phi_\alpha = 1.0$;
XCI	$u_1^0 = 0.3$; $u_2^0 = 2.0$; $D_{u_1} = 0.05$; $D_{u_2} = 10.0$; $\delta = 1.15$; $\kappa_0 = 0.067$; $K = 1.0$; $\gamma_0 = 1.0$; $\kappa = 0.5$; $n = 4$; $\alpha_{u_1} = \alpha_{u_2} = 0.05$; $\beta_{u_1} = \beta_{u_2} = 0.1$
Tethering	$n_{\text{thr}} = 100$; $n_{\text{total}} = 20000$; $\phi_{\text{thr}} = 0.005$

Table S5: Parameters used to obtain results of differential diffusivity of the XIST molecules in one spatial dimension (Figure S10 and S11 in Supplementary File)

Parameters (units arbitrary)
$\Delta x = 0.2; \Delta t = 10^{-3}; L = 80; \text{Boundary} = \text{Zero-Flux}$
$[XIST]_0 = 0; n_\beta = 4; n_\alpha = 4; \alpha_X = 0.01;$ $\beta_X = 1.0$ (for $x = 40$) and $= 0.0$ (otherwise); $\phi_\beta = 0.1; \phi_\alpha = 1.0;$
$u_1^0 = 0.3; u_2^0 = 2.0; D_{u_1} = 0.025; D_{u_2} = 10.0; \delta = 1.10;$ $\kappa_0 = 0.067; K = 1.2; n = 4; \alpha_{u_1} = \alpha_{u_2} = 0.05;$ $\beta_{u_1} = \beta_{u_2} = 0.1;$ $\gamma = \begin{cases} 1 & \text{for } 4 \leq x \leq 20 \text{ and } 60 \leq x \leq 76 \\ 0 & \text{Otherwise} \end{cases}$
$n_{\text{thr}} = 10; n_{\text{total}} = 400; \phi_{\text{thr}} = 0.5$
S10(A) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 2.5$ S10(B) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 5$ S10(C) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 7.5$ S10(D) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 10$ S10(E) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 15$
S11(A) $D_X^{\text{void}} = 2.5, D_X^{\text{chr}} = 10$ S11(B) $D_X^{\text{void}} = 5, D_X^{\text{chr}} = 10$ S11(C) $D_X^{\text{void}} = 7.5, D_X^{\text{chr}} = 10$ S11(D) $D_X^{\text{void}} = 10, D_X^{\text{chr}} = 10$ S11(E) $D_X^{\text{void}} = 15, D_X^{\text{chr}} = 10$

Table S6: Parameters used to implement imposed tethering in one dimension (Depicted in Figure S14 of the Supplementary File)

Parameters (units arbitrary)
$\Delta x = 0.2; \Delta t = 10^{-3}; L = 80; \text{Boundary} = \text{Zero-Flux}$
$[XIST]_0 = 0; n_\beta = 4; n_\alpha = 4; \alpha_X = 0.01;$ $\beta_X = 1.0$ (for $x = 40$) and $= 0.0$ (otherwise); $\phi_\beta = 0.1; \phi_\alpha = 1.0;$
$u_1^0 = 0.3; u_2^0 = 2.0; D_{u_1} = 0.025; D_{u_2} = 10.0; \delta = 1.10;$ $\kappa_0 = 0.067; K = 1.2; n = 4; \alpha_{u_1} = \alpha_{u_2} = 0.05;$ $\beta_{u_1} = \beta_{u_2} = 0.1;$ $\gamma = 1.0, D_X = 10.0$

Table S7: Performance statistics for 20 model realizations with random XIST tethering sites

	Sensitivity	Specificity	False Positive Rate	Precision	Accuracy
Mean	0.3342	0.8102	0.1898	0.1357	0.7716
Standard Deviation	0.0841	0.0493	0.0493	0.0196	0.0400
Minimum	0.1842	0.7163	0.1372	0.0833	0.6966
25 Percentile	0.2632	0.7948	0.1512	0.1255	0.7580
Median	0.3026	0.8256	0.1744	0.1345	0.7831
75 Percentile	0.4013	0.8488	0.2052	0.1447	0.8034
Maximum	0.4737	0.8628	0.2837	0.1765	0.8141

Table S8: Confusion matrix aggregated across 20 model realizations with random XIST tethering sites

	Escapees observed	Silenced genes observed
Escapees predicted	254	1632
Silenced genes predicted	506	6968

Table S9: Compiled table of parameters used in different modules with brief explanation

Parameter	Description / Scope	Value	Unit	Brief Explanation
Δt	Time step size	10^{-3}	a.u.*	The non-dimensionalization leads to equality of 1 t.u.= 0.0144 secs
Δx	Spatial step size (Grid spacing)	0.2 (0.5 for 3D module)	a.u.	approximate convergence criteria $D \frac{\Delta t}{(\Delta x)^2} \leq \frac{1}{2}$ (for 1D) and $\leq \frac{1}{6}$ (for 3D)
L	System length (1D domain)	20 (S5A), 40 (S5B, S8), 80 (S3, S5, S6)	a.u.	Arbitrarily chosen for prototypical study
L_x, L_y, L_z	System dimensions (3D domain)	$L_x = 70$; $L_y = 72.5$; $L_z = 70.5$	a.u.	Consequence of chromosome structure inferred from Hi-C data
u_1^0	Initial concentration of component u_1	0.3 (0.2683 for Fig. S8)	a.u.	Determined by bi-stability
u_2^0	Initial concentration of component u_2	2.0	a.u.	Determined by bi-stability
D_{u_1}	Diffusion coefficient of component u_1	0.025 (0.05 for 3D module)	a.u.	1 a.u.= $1.36 \times 10^{-5} \mu\text{m}^2 \text{sec}^{-1}$ and the rationale is given in Supplementary Data
D_{u_2}	Diffusion coefficient of component u_2	10.0	a.u.	same as D_{u_1}
δ	Kinetic parameter	1.10 (1.20 for Fig. S8, 1.15 for 3D module)	a.u.	ensuring bi-stability condition
κ_0	Kinetic parameter	0.067	a.u.	ensuring bi-stability condition
K	Activation/Inhibition threshold constant	1.0 (1.1 for Fig. S8, 1.2 for integrated/1D modules)	a.u.	ensuring bi-stability condition
n	Hill coefficient / Cooperativity index	4	NA	High degree of non-linearity ensure high sensitivity and clear boundary between active and inactive regions
$\alpha_{u_1}, \alpha_{u_2}$	Degradation rate constants for u_1, u_2	0.05 (0.06 for Fig. S5D)	a.u.	The relative ratio between production rate [1] and degradation rate [2] is maintained which agrees with the experimental findings
β_{u_1}, β_{u_2}	Production/Basal rate constants for u_1, u_2	0.1	a.u.	see degradation rate constant's explanation
n_{stim}	Number of stimulus pulses	1	NA	arbitrarily chosen for prototypical study

Continued on next page

Table S9: Compiled table of parameters used in different modules with brief explanation

Parameter	Description / Scope	Value	Unit	Brief Explanation
t_1, t_2	Stimulus duration interval	$t_1 = 2; t_2 = 4$	a.u.	arbitrarily chosen for prototypical study
γ	Chromatin state / Interaction modifier	1.0 (domain-specific variations in Fig. S5 & S8)	NA	Highly sensitive parameter used to switch between monostability and bi-stability
$[XIST]_0$	Initial concentration of XIST molecules	0	a.u.	arbitrarily chosen
D_X	Diffusion coefficient of XIST molecules	1.0 (25.0 for 3D module, 10.0 for imposed tethering)	a.u.	$D_X = 1.0 \text{ a. u.} = 1.36 \times 10^{-5} \mu\text{m}^2\text{sec}^{-1}$ which is well below the lower bound of lighter m-RNA molecules [3]
α_X	Basal decay/production rate constant of XIST	0.01	a.u.	fixed in comparison to other degradation rate constants
β_X	Production rate constant of XIST	1.0 at $x = 40$ (10.0 for XIC in 3D module); 0.0 otherwise	a.u.	fixed in comparison to other production rate constants
n_α, n_β	Cooperativity coefficients for XIST regulation	4	NA	same as n
ϕ_α	Scaling parameter	1.0	a.u.	arbitrarily chosen
ϕ_β	Scaling parameter	0.1 (0.01 for 3D module)	a.u.	arbitrarily chosen
n_{thr}	Number of tethering sites per domain	10 (100 for 3D module)	NA	reported experimentally
n_{total}	Total number of elements/particles modeled	400 (20,000 for 3D module)	NA	chosen to implement a reasonable resolution of gene locations
ϕ_{thr}	Tethering efficiency/probability parameter	0.5 (0.005 for 3D module)	a.u.	arbitrarily chosen
D_X^{void}	XIST diffusion coefficient in void regions	10.0 (2.5 to 15.0 across S10 & S11 sweeps)	a.u.	consistent with D_X
D_X^{chr}	XIST diffusion coefficient in chromatin regions	10.0 (2.5 to 15.0 across S10 & S11 sweeps)	a.u.	significantly lower than D_X^{void} based on physical principle

*a.u. = arbitrary units; NA = Not Applicable / Dimensionless

References for Table S9

- [1] Guido van Mierlo, Gert Jan C. Veenstra, Michiel Vermeulen, and Hendrik Marks. The complexity of prc2 subcomplexes. *Trends in Cell Biology*, 29(8):660–671, August 2019.
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- [3] Marlene Oeffinger and Daniel Zenklusen. To the pore and through the pore: A story of mrna export kinetics. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1819(6):494–506, 2012.