Supplementary material:

1- **Modeling PA experiments**

a/ **Standard model**

The dynamics of chromatin interacting proteins can be modeled in euchromatin and in heterochromatin using partial differential equations (PDE), which for single-step reactions read:

\[
\begin{align*}
\frac{\partial A}{\partial t} &= D\Delta A - k_1 \times C(r) \times A + k_2 B \\
\frac{\partial B}{\partial t} &= k_1 \times C(r) \times A - k_2 B
\end{align*}
\]

(S1)

\(A, B\) and \(C(r)\) represent pools of free, or bound proteins, and the chromatin concentration, respectively. Note that measured intensities are sums of \(A+B\). \(D\) is the diffusion coefficient. \(k_1\) and \(k_2\) are the on and off rates of the binding reaction, respectively. These reaction parameters may take different values in heterochromatin vs. euchromatin.

PDE can be solved taking the complex cellular geometry into account using Berkeley Madonna (see (Beaudouin et al., 2006) for detailed explanations on 2D solutions). This computation requires several inputs including steady-state nuclear intensity distributions, the first image acquired right after PA, and measurement volumes in euchromatin and heterochromatin (Fig. S3c). The grid size was set to 450*450 nm. Interestingly, because PA experiments are performed at steady state, several simplifications have to be considered. First, as demonstrated in (Beaudouin et al., 2006), PDE imply that the fraction of freely diffusing proteins (Free) is homogeneous at steady state. Second, the association reaction term \(k_1 \times C(r)\) can be expressed as a function of \(k_2\) and Free (Beaudouin et al., 2006):

\[
\begin{align*}
\frac{i^{ss}(r)}{\langle i^{ss}\rangle} = \frac{k_1 \times C(r)}{k_2 \text{Free}}
\end{align*}
\]

(S2)

with \(i^{ss}(r)\) the protein steady-state intensity, and \(\langle i^{ss}\rangle\) the mean value over the nucleus. Free and \(k_2\) can be measured based on the fit of euchromatin relaxation curves (e.g. Fig. 3c).

Notably, confocal sectioning effects can also be considered by implementing 3D solutions, and, in this case, the cellular geometry was simplified with one single heterochromatin focus and rectangular edges (Fig. S3c).

We emphasize that this approach underlies several implications, which contradict experimental results. First, diffusion is homogeneous according to standard PDE, unless obstacles are considered phenomenologically by introducing heterogeneous diffusion coefficients in Eqn. S1 (see more below). Second, steady state concentration variations of inert tracers, which are detected in crowded compartments (see e.g. Fig. 1a), are inconsistent with the expected homogeneity of Free. In addition, standard PDE provide poor fits for heterochromatin relaxations (Fig. 3c).

b/ **Improved PDE**
Because crowding is expected to be associated with an enhancement of association reactions, we assumed that binding parameters in Eq. S1, namely $k_1$ and $k_2$, were different in heterochromatin and euchromatin as a first level of analysis. However, it is impossible to explore the whole ($k_1, k_2$) parameter space because these two parameters are linked at steady state according to Eq. S2. In Fig. S3e, we compare the fit obtained with a homogeneous model, i.e. similar reaction constants in eu- and heterochromatin (black curves), and with an heterogeneous model (yellow curves). This refined model did not allow us to obtain two steps kinetics with slowed down relaxation onsets.

Second, we considered that crowding effects occurred, and assumed that diffusion was two-fold slowed down in dense compartments. This feature was introduced in Eq. S1, based on a specific heterochromatin grid (Fig. S3c). Note that this approach is phenomenological because, to our knowledge, there is no exact computation that includes crowding effects in heterogeneous environments. In addition, this model does not account for variations of Free. This refinement was insufficient to improve fits of heterochromatin relaxation curves (Fig. S3f).

c/ Molecular dynamics simulations of random networks

2D molecular dynamics simulations were implemented using a method inspired from (Schnell and Turner, 2004), yet simplified: rectangular grids were randomly filled with proteins, binding sites or obstacles. We assumed that obstacles, binding sites (occupied or not) and proteins had the same size. Dynamics were governed by the following rules. We assumed that there was no interaction between diffusing proteins, and that tracers followed isotropic random motions. Tracers colliding with obstacles remained in their previous position. When tracers reached one available binding site, an association event was triggered with 0.5 probability. We considered that occupied sites were obstacles for diffusing proteins. Dissociation was characterized by an escape probability $P_{off}$ that could be adjusted to control steady state free pools. We could tune the fraction of obstacles from 0% to 100% in order to investigate molecular crowding consequences. We could also adjust the number of diffusing proteins from 0.15 to 0.9 molecule per binding site, i.e. assuming they were less abundant than their ubiquitous binding sites. Each simulation started with an equilibration procedure: the initial distribution of tracers was random, and the system was allowed to relax to steady state during $10^6$ time steps. Starting from this relaxed situation, tracers localized in defined regions were highlighted, and .png images were extracted to monitor heterochromatin or euchromatin redistribution kinetics, which were quantified using ImageJ.

The fraction of obstacles plus binding sites in euchromatin is unknown. Nonetheless, the diffusive hindrance is linked to the density of obstacles (Saxton, 1994), and simulations showed that $D/D_0 \sim 1/3$ corresponds to an obstacle fraction of $\sim 30\%$. Because heterochromatin is typically two-fold denser than euchromatin, its density was set to 60%. This fraction of obstacles is above the percolation threshold in 2D, and heterochromatin should be fully inaccessible under these conditions. Thus, diffusion simulations, i.e. without binding sites, were run with eu- and heterochromatin densities of 25% and 50%, respectively, and diffusion+binding simulations with densities of 30% and 60%.

When comparing to the fractal model, chromatin density appears to be relatively low (Table 1). This discrepancy stems from two approximations of this
random nuclear model. First, the nucleus is viewed as 2D organelle, and we expect
the density of obstacles necessary to obtain a diffusive hindrance of 1/3 to be higher
in 3D. Second, chromatin is not an immobile structure, and the presence of mobile
obstacles tends to reduce the diffusive hindrance (Saxton, 1994). Hence, a realistic
3D chromatin model should be relatively consistent with the fractal model in terms of
chromatin accessible volume.

d/ Fractal kinetics
We tested the possibility that fractal kinetics, i.e. kinetics with time dependent
reaction constants, occurred in the nucleus (see Eqn (1) in main text). One
problematic aspect of fractal kinetics is the clear breakdown of the formalism as time
decreases to 0 for \( \varepsilon > 0 \), this breakdown being incompatible with finite element
modelling. We thus proposed to use the Zipf-Mandelbrot formulation (Schnell and
Turner, 2004) for reaction constants:

\[
k(t) \approx \frac{k}{(1 + \frac{t}{t_0})^\varepsilon}
\]  

(S3)

With \( k \) the rate constant at \( t=0 \), \( t_0 \) the characteristic time scale at which
the kinetics become fractal, and \( 0 \leq \varepsilon \leq 1 \). For diffusive motions in fractal environments,
\( \varepsilon = 1 - ds/2 \) with \( ds \) the spectral dimension, which is defined by \( ds = \gamma f \), and \( ds \leq 2 \). For
\( ds > 2 \), \( \varepsilon = 1 \). Notably, the vast majority of fractal structures formed by polymer
solutions are characterized by compact exploration, i.e. \( ds \leq 2 \) (Cassi and Regina,
1993).

The occurrence of fractal kinetics is related to anomalous diffusion and to the
architecture in which tracers diffuse. In the finite element model, one pixel
corresponds to 450 nm*450 nm, which is larger than chromatin upper limit of self-
similarity (~100 nm). Hence, in the short time limit, proteins located in one pixel of
our PDE model explore a fractal environment in which Eq. S3 is valid.

Moreover, because dissociation only depends on the stability of protein
complexes, whereas association events can be severely limited or favored depending
on chromatin organization (Zimmerman and Minton, 1993), we assumed that only
association reactions were fractal.

Importantly, fractal kinetics are associated to three fitting parameters, namely
\( k, t_0 \) and \( \varepsilon \). \( t_0 \) was systematically set to the time step of PA experiments (\( t_{samp} \)), and
we checked that \( \varepsilon \) fitted values did not depend on \( t_0 \) for \( t_0 < t_{samp} \) (data not shown). \( k_1 \)
instead strongly varies with \( t_0 \) (data not shown), and we did not analyze
quantitatively this parameter.

2- Physics laws for fractal model predictions
We first used the relationship linking the fractal dimension \( f \) to the accessible
volume to total volume ratio, which is valid for fractals with constant fractal
dimensions in the self-similarity domain (Puzenko et al., 1999):

\[
\Phi = \left(\frac{h}{H}\right)^{3-f}
\]  

(S4)

with \( H \) and \( h \) the upper and lower limit in self-similarity, respectively.
Second, we used the Neale and Nader formalism in order to estimate the diffusive hindrance in chromatin (Neale and Nader, 1974):

\[
\frac{D}{D_0} = \frac{2 \times \Phi}{3 - \Phi}
\]  

(S5)

For the sake of simplicity, we used this approximated relationship, although several refined formulations have been proposed.

Finally, in an entangled polymer solution, the upper limit of self-similarity is defined by the maximum pore size, and it varies with the polymer concentration following a universal behavior (de Gennes, 1979):

\[
H \propto c^{-\frac{3}{4}}
\]  

(S6)

Hence, assuming that chromatin polymeric structure is roughly the same in heterochromatin and in euchromatin, we deduce that \( H_{\text{heterochromatin}} / H_{\text{euchromatin}} \approx 0.6 \) for a two-fold enriched heterochromatin focus.

**Figure S1:** Measuring Heterochromatin exclusion. (a-b-c) 25 kDa dextran, 160 kDa dextran and 500 kDa dextran exclusion in heterochromatin vs. euchromatin of NIH3T3 cells is plotted as a function of heterochromatin to euchromatin density ratio, which was inferred from the Hoechst pattern (blue, purple and black, respectively). At the resolution of optical microscopy, exclusion appears to increase linearly with heterochromatin concentration, and a single parameter linear fit can be performed to deduce the exclusion rate of each marker (solid lines). Exclusion rates are \(-0.06 \pm 0.01\), \(-0.09 \pm 0.01\) and \(-0.11 \pm 0.02\) for 25 kDa dextran, 160 kDa dextran and 500 kDa dextran, respectively. (d) The different linear exclusion rates are represented, and the green vertical line corresponds to a heterochromatin density of 6, as highlighted by the green arrowhead in Fig. 1b. (e) Left: Hoechst pixel intensity distributions of the control and Suv39H1 overexpressing cells represented in Fig. 1c (blue and purple, respectively). Right: Pixel intensity distributions are fitted with two Gaussians, one adjusted to low intensity pixels, i.e. related to low DNA density regions, the other to high intensity pixels, i.e. to high DNA density regions (red and green curves, respectively). Phenomenologically, the 2 Gaussians can be related to euchromatin and heterochromatin, and one can thus compute the number of heterochromatin and euchromatin pixels. The vertical dotted line indicates a pixel intensity of 135, i.e. the transition between red and green pixels in thresholded images in Fig. 1c. Scale bars 5 µm.

**Figure S2:** Size dependence of heterochromatin and nucleolar diffusive properties. (a) NIH3T3 cells transiently expressing H2b-mRFP and mEGFP-2 subjected to FCS measurements. Red and green crosses on the H2b image indicate positions in euchromatin and heterochromatin, respectively, where measurements were performed. The right image was acquired after FCS in order to assess the precision of the experiment (red and green circles, respectively). Note that this quality control evaluation was systematically performed, and that the size of
heterochromatin foci is at the limit of the FCS spatial resolution, leading to large variations in the measurements. Graphs show renormalized ACFs obtained in euchromatin (red), heterochromatin (green). Fits were performed with an anomalous diffusion model (solid curves) showing a drop of the mEGFP-2 residence time from 460 µs in euchromatin to 910 µs in heterochromatin, and anomalous coefficients of 0.77 and 0.75, respectively. Scale bar 10 µm. (b) Left: crude extract of NRK cells expressing 1, 2, 5 and 10 mEGFP arrays (lanes 6, 5, 4 and 3, respectively) were subjected to electrophoresis and immunoblotted together with commercial purified GFP (lane 2). Figures along the vertical axis correspond to MWs in kDa. Several bands appear in mEGFP-10 and mEGFP-5 lanes (the intensity distribution in the region outlined with a red frame is plotted in the upper graph), showing that these arrays are partly degraded in cells. The weakly zoomed image represents NRK cells expressing the mEGFP-10 array. Cell-to-cell variability in the nucleoplasmic to cytoplasmic concentration ratio is observed, which is related to the fact that the passage of large tracers through nuclear pores is limited by the size exclusion of their central channel, thereby inducing an accumulation after translation in the cytoplasm. Scale bar 50 µm. We then picked three NRK cells coexpressing mEGFP-10 and H2b-mRFP. mEGFP-10 protein is abundant, slightly excluded and significantly excluded from the nucleus as compared to the cytoplasm in cells 1, 2 and 3, respectively. Scale bar 10 µm. The right panel shows normalized ACF measured in the nucleoplasm of cells 1, 2 and 3; the residence time derived from the anomalous diffusion fitting is 295 µs, 690 µs and 1070 µs in cells 1, 2 and 3 respectively, showing that it increases with mEGFP-10 apparent nuclear exclusion. A large variability for high MW mEGFP arrays is thus observed in a cell population, but we can not conclude on the degree of degradation of these probes at the level of individual cells. (c) The graph at the left shows the nucleolar to nucleoplasmic residence time ratio vs. nucleoplasmic residence times of mEGFP, mEGFP-2, mEGFP-5 and mEGFP-10 tandems. The solid line is a linear fit, which shows that the increase in diffusive hindrance with molecular weight is more rapid in nucleoli than in the nucleoplasm. The black arrowhead indicates the FCS response obtained in Fig. 2a. The graph at the right indicates that the nucleolar FCS anomaly parameter tends to decrease with molecular weight. (d) The graph at the left shows the heterochromatin to euchromatin residence time ratio vs. euchromatin residence time, based on the response of GFP dimers, pentamers and decamers. The bold line is a linear fit to the data that shows no significant variation in diffusive hindrance with MW. The graph at the left displays heterochromatin and euchromatin anomaly parameters as a function of euchromatin residence time (black crosses and blue diamonds, respectively). Our measurements thus indicate that heterochromatin diffusion slow down and anomaly parameter are size independent.

**Figure S3:** PA calibration and analysis. (a) Photoactivated spots calibration: PA was performed in NRK cells expressing H2b-PAGFP, which is immobile for several hours (Beaudouin et al., 2006). In the left panel, 3D pseudo-color reconstruction of the photoactivated volume was done by thresholding. In the right panel, graphs show mean intensities along the axial or equatorial axis (blue squares) with their corresponding Gaussian fits (solid lines). (b)
Experiments are carried out in two steps. First, local PAs are performed with ~10 ms acquisition rates. Then, the nucleus is globally activated with unchanged acquisition settings. This second experiment enables us to monitor PAGFP photophysics independently of the dynamics of interacting protein. Scale bar=10 µm. (c) The left graph shows the raw normalized heterochromatin intensity data and the normalized intensity data after photoactivating the whole nucleus (blue and black datasets, respectively). Note that we observe an increase in intensity right after whole nucleus photoactivation, which is an intrinsic photophysics property of PAGFP. The right plot represents heterochromatin curve compensated for PAGFP photophysics obtained by dividing the redistribution kinetics (blue dataset in the left graph) by the whole nucleus response (black dataset in the right graph). Heterochromatin relaxation appears to be characterized by a two step kinetics with a plateau at short time scales (purple arrowhead) associated to crowding mediated trapping in this dense compartment (see main text). (d) The left panel shows the inputs required for fluorescence redistribution simulations. Eu- and Heterochromatin measurement volumes are represented with red and green circles, respectively. The right panel depicts 3D PDE geometry with one heterochromatin focus characterized by a 3-fold enriched chromatin concentration. Experimental conditions are reproduced bona fide by measuring redistribution kinetics in confocal slices which thickness matches that of heterochromatin (gray planes). (e) 2D PDE modeling applied to a typical RCC1 response curve in heterochromatin (green) and euchromatin (red). Fitting is performed with Berkeley Madonna assigning homogeneous or heterogeneous binding constants in heterochromatin (black and orange solid curves, respectively). Interestingly, $k_1$ should remain constant in heterochromatin (see graph annotation), in contradiction with crowding predictions. In addition, tuning $k_2$ does not suffice to increase fitting accuracy particularly at short timescales (the inset shows the residual of the fit to the data for euchromatin (red dataset), and heterochromatin using a homogeneous or heterogeneous distributions of binding sites (black and orange, respectively). (f) Dynamics of tracers within heterochromatin according to the 3D geometry depicted in d including a two-fold diffusive hindrance in heterochromatin. The free pool is kept constant at 5%, and the dissociation rate $k_2$ is progressively decreased. Relaxations become slower with long lived interactions, but two steps kinetics can never be observed with this model.

**Figure S4:** Random models for nuclear protein dynamics. (a) The nucleus is defined as a 2D randomly crowded organelle containing membraneless compartments in which static and uniform macromolecules are placed at random with a defined density. These macromolecules generate a network of obstacles, in which fluorescent tracers diffuse, as in e.g. (Saxton, 1994). Then, the density of obstacles had to be determined for each compartment and fed into the model. The diffusive hindrance depends on the concentration of obstacles (Saxton, 1994), and, given that D/D0=0.3, we deduce from simulations that euchromatin contains an obstacle fraction of ~30%. Chromatin concentration is typically two-fold enriched in heterochromatin (upper panel), leading a density of 60% that is above the percolation threshold in 2D. Hence, we decided to run fluorescence
distribution simulations of half nucleus PA with densities of obstacles of 25 % and 50 % in eu- and hetero-chromatin, respectively. (b) We observed that a smaller amount of diffusive markers could enter in crowded regions (left panel), while uptake kinetics were very similar in crowded and uncrowded compartments (right panel). (c) Although concentration variations occur at steady state (left panel), the compensated concentration, defined by the number of particles per unit of obstacle-free space, is homogeneous in low and high density compartments (right panel), as computed for 25% and 38% obstacles densities. This suggests that if the apparent concentration, i.e. the number of particles per unit of total space, exhibits strong variations due to volume exclusion, the compensated concentration is uniform in the nucleus and represents the relevant parameter for diffusion. (d) Molecular dynamics simulations with 30% and 60% obstacles plus binding site fractions in eu- and heterochromatin, respectively (upper left panel). Redistribution kinetics were monitored in heterochromatin (green set of curves) and euchromatin (red set of curves). We first ran a reference simulation with no obstacles (dark red and dark green curves). Then, the obstacle-to-binding site ratio was increased from 30%, 60% up to 80%, and the corresponding datasets are represented with brighter colors for increasing obstacle-to-binding site ratios. Note that the tracer to binding site fraction was set to 0.2 in this case but we checked that this parameter did not change our conclusions, and the fraction of freely diffusing tracers to ~15%, corresponding to escape probabilities $P_{off}$ of 0.995, 0.994, 0.996 and 0.998 in obstacle free, 30%, 60% and 80% simulations. Redistribution kinetics were compared in heterochromatin and euchromatin for different binding site to obstacle ratio, and we observed that heterochromatin relaxations were globally slowed down due the diffusive hindrance induced by obstacles, and we could not observe two-step kinetics.

**Figure S5:** Controls in single QDs tracking. (a) Histogram of QDs diffusion coefficient in live cells deduced from the fitting of displacement histograms at 30.6 ms (see Fig. 3b in main text). QDs microinjected in nuclei tend to form aggregates, leading to heterogeneous populations of tracers. Notably, due to their small sizes, single QDs diffuse very rapidly across the nucleus, and they cannot be accurately tracked at 2 ms acquisition rate. Instead, aggregates characterized by a nuclear diffusion coefficient of $D\sim 0.5$ $\mu$m$^2$/s are well-suited for tracking due to their brightness and their slow diffusion. Although tracers are heterogeneous, their behaviors can be compared in displacement histograms by rescaling displacements $(L)$ with $1/\sqrt{D}$, which is invariant on the probe size at fixed times. (b) Single QD displacement histograms at various fixed times are plotted (cyan datasets), and fits based on the Brownian diffusion model are appended ((Saxton, 1993), solid curves). Displacements histograms are strongly anomalous for mean displacements typically smaller than 100 nm (dotted line). Note that the fitted diffusion coefficient tends to decrease with time (not shown). (c) Displacement histograms at 1.9 ms and 30.6 ms for QDs freely diffusing in water, and their respective fits to a brownian diffusion model. Expectedly, their behavior is in agreement with the standard diffusion model. (d) Plots of the ratio of displacement histograms at 1.9 ms vs. 7.8 ms (cyan dataset), and 7.8 ms vs. 30.4 ms (blue dataset), and their corresponding fit with...
a stretched exponential model, that expectedly indicates a fractal dimension of 3, given that $\gamma=0.91$ (data not shown). (e-f) QDs bound to chromatin or to coverslips are tracked at 1.9 ms, and distributions of displacements are plotted after 1.9 ms and 30.6 ms. Distributions fit to a standard Gaussian model. (g) We tested whether long tailed histograms obtained for QDs nuclear diffusion could be associated with transient binding to chromatin. Left: we assume that tracked QDs can either be bound to chromatin or diffusing freely. These two states are associated with two standard Gaussians in the $\Delta t=1.9$ ms histogram, one containing rather long diffusive steps, and the other one containing short steps which characteristic length scale depends on the tracking accuracy. This double Gaussian model can be used to fit experimental histograms (green curve, the blue one corresponding to standard Brownian model). The fraction of immobile steps, the tracking accuracy and the diffusion coefficient during diffusive periods can be extracted. Using these three parameters, we could then implement Brownian trajectory simulations as in (Huet et al., 2006). Right: Step histograms at $\Delta t=30.6$ ms obtained from the simulation (green) and in experiments (black). The difference in breadth shows that QDs anomalous behavior cannot be explained by transient binding to chromatin.


Fig. S1

a. Graph showing Heterochromatin/Euchromatin concentration against heterochromatin concentration for Dextran-25.

b. Graph showing Heterochromatin/Euchromatin concentration against heterochromatin concentration for Dextran-160.

c. Graph showing Heterochromatin/Euchromatin concentration against heterochromatin concentration for Dextran-500.

d. Graph showing Heterochromatin/Euchromatin concentration against heterochromatin concentration for Merge.

e. Graph showing number of pixels against pixel intensity for blue: NRK control cell and purple: NRK expressing Suv39H1.
H2b-mRFP in NIH3T3 cell

After FCS

Normalized autocorrelation

Count rate (kHz)

Nucleolar/Nucleoplasmic residence

Nucleoplasmic residence time (µs)

Nucleolar anomaly parameter

Euchromatin residence time (µs)

Hetero/euchromatin residence

Euchromatin residence time (µs)

Normalized Autocorrelation Function

Residence time (µs)

Normalized autocorrelation

Count rate (kHz)

Normalized autocorrelation

Residence time (µs)

Normalized autocorrelation

Residence time (µs)

Normalized autocorrelation

Residence time (µs)
Steady state t=0 s

Local PA

Global PA

Steady-state Heterochromatin definition

Initial conditions

Heterochromatin definition

Euchromatin, Free=5%

Fits with homogeneous binding constants \( k_1 = 220 \text{ mol/s}, k_2 = 15 \text{ s}^{-1} \)

Fits with heterogeneous binding constants - euchromatin: \( k_1 = 220 \text{ mol/s}, k_2 = 15 \text{ s}^{-1} \) - heterochromatin: \( k_1 = 220 \text{ mol/s}, k_2 = 2.5 \text{ s}^{-1} \)
a Random nuclear architecture model for diffusion

Steady state

Relaxation

b Relaxation kinetics

Intensity (au)

Normalized intensity

Time steps (*10^3)

c Steady state compensated concentration measurements

Intensity (au)

Time steps (*10^3)

0 5 10 15 20 25

0 1 2 3

10 20 30 40

0.0 0.4 0.8

0 200 400 600 800 1000

0.0 0.2 0.4 0.6 0.8 1.0

0 5 10 15 20 25

0 10 20 30 40

0.0 0.2 0.4 0.6 0.8 1.0

d Random model for diffusion+interaction dynamics

obstacle/binding sites

Renormalized intensity

Time steps (x10^5)
Anomalous behavior is not due to transient binding