In this chapter, we discuss central results from our model which follow from considering chromosomes as active matter. In order to do that we compare our results with experimental data which are extracted numerically from Fig. 6((h): right column) of Ref. [36] (shown in Fig. 1.10(a)) for chromosomes 18 and 19 and from Fig. 6((g): left column) of the same reference (shown in Fig. 1.10(b)) for chromosomes 12 and 20; these data originate in Refs. [90, 91]. These are plotted using open symbols in chromosome specific colours (see figure captions), when displayed.

From an ensemble of steady state configurations, we evaluate a number of structural parameters and distribution functions for our model chromosomes. We generate model predictions for all chromosomes but concentrate here on the pairs 18/19 (Chromosome 19: 62.03 genes/Mb and 60 Mb size, Chromosome 18: 18.64 genes/Mb and 78 Mb size) and 12/20 (Chromosome 12: 30.92 genes/Mb and 134 Mb size, Chromosome 20: 29.71 genes/Mb and 63 Mb size) for specificity. Note that Chromosomes 18 and 19 are similar in size but differ in gene density, while chromosomes 12 and 20 differ in size but have comparable gene densities, making them ideal candidates to test for segregation by gene density. We also measure local activity distributions in steady state at radial distance $R$, by averaging active temperatures associated with all monomers in a small range about $R$. In our simulations of the ellipsoidal case, we compute similar quantities, scaling our distribution functions to obtain densities as a function of an effective radial coordinate. Fig. 4.2, 4.3, 4.4, 4.5 and 4.6 contain results for the model without loops, whereas Fig. 4.7 and 4.8 are for the general random loop model with inhomogeneous activity.
Our main results are as follows.

- Our model predicts chromosome positioning across a variety of conditions.
- Chromosome positioning is unstructured if non-equilibrium activity is absent or uniform.
- Purely geometrical confinement arising from nuclear shape is a relatively minor determinant of positioning.
- Allowing specific interactions of even a small number of monomers with the nuclear envelope strongly modulates positioning.
- The dynamical evolution of chromosome positions upon perturbation can be tracked in our model.
- Chromosome territories emerge from the combination of compact configurations for individual chromosomes and activity-based segregation.
- Our model can also predict, in some regimes, size dependent segregation.

We discuss each of these results in turn below. In the next section, we describe our results of chromosome positioning in different geometries; sphere, oblate and prolate. The question of how chromosome positioning is inverted, allowing specific interactions of small number of monomers with the nuclear envelope, is taken up in Section 4.2. In Section 4.3, we show that combination of compact configurations for individual chromosomes, constructed by using the random loop model, and activity-based segregation is sufficient to generate the chromosome territories. Finally, we show that our model can also predict size dependent segregation of chromosomes in some limits.

### 4.1 Chromosome positioning in spherical and ellipsoidal nuclei

The sequence of figures in Fig. 4.1(A) – (C), exhibit illustrative snapshots of chromosomes in our model under a variety of different conditions. We model different nuclear shapes, vary the large-scale configurations of individual chromosomes, as well as study both equilibrium and non-equilibrium situations. Fig. 4.1(A) – (C) shows
4.1 Chromosome positioning in spherical and ellipsoidal nuclei

Figure 4.1: Configurational snapshots showing positions of simulated human chromosome 18 (red) and chromosome 19 (blue) for our model, in the background of other chromosomes (grey), for (A) and (C), spherical nuclei, and (B), a prolate ellipsoidal nucleus. The surface configuration of monomers, colour coded by chromosome, are shown in (D) and (E).

Chromosomes 18 (red) and 19 (blue) within the background of other chromosomes, shown in grey scale background. These are displayed in Fig. 4.1(A), for a simulated spherical nucleus and in Fig. 4.1(B) for a simulated prolate ellipsoidal nucleus. The simulations in Fig. 4.1(A) and (B) are for thermal equilibrium, with $T_a = T_{eq}$ for all monomers. Chromosomes contain no permanent loops in Fig. 4.1(A) and (B) where chromosomes are displaying substantial intermingling.

In contrast to these, Fig. 4.1(C) shows configurations for our non-equilibrium model with inhomogeneous activity. Here, following the random loop model, we assign
individual chromosomes a fixed, small density of loops of arbitrary sizes, thus ensuring that the resulting chromosome configurations are compact. The results for this case reflect ‘activity-based segregation’, in which bulk intermingling is considerably suppressed, a territorial organization emerges, and the gene-dense chromosome 19 occupies a more interior position than the gene-poor chromosome 18.

In addition, Fig. 4.1(D) – (E) display the surface configuration of monomers, both for the inhomogeneous activity case. A configurational snapshot with non-compact chromosome configurations (no loops) is shown in Fig. 4.1(D).

Fig. 4.1(E) provides a snapshot for the case of compact chromosome configurations, generated through the random loop model described above. As can be seen by comparing Fig. 4.1(D) – (E), activity-based segregation alone does not give rise to chromosome territories if there is no mechanism to create compact chromosome configurations. Inducing such compactness, by the expedient of allowing for a small density of random connections along each chromosome, allows chromosomes to segregate by activity as well as reduces intermingling. The surface configurations of Fig. 4.1(E) provide striking visual evidence for a territorial organization of chromosomes in the random loop model coupled to inhomogeneous activity. The nuclear envelope is represented by a repulsive, short-ranged potential, confining chromosomes to a given geometry in all these cases.

Fig. 4.2(A)(i) – (iii) shows cut-away spheres displaying the time-averaged local activity at different points within the spherical nucleus. Activity is measured in units of the thermodynamic temperature, colour-coded with magnitude and further scaled to the interval [0:1] as shown. (The colour code thus corresponds to the interval between the thermodynamic temperature $T_{eq}$ and the active temperature assigned to monomers with a high density of active genes, $T_a = 20T_{eq}$, with the darkest shades representing $T_{eq}$ and the lightest shades $T_a$.) Given the relationship we assume between activity and gene density, a uniform colour indicates that gene densities are uniform. Fig. 4.2(A)(i) represents such distributions in thermal equilibrium, while Fig. 4.2(A)(ii) represents the case where all monomers are assigned a uniformly high activity temperature of $T_a = 20T_{eq}$. The distribution of activity is unstructured in both cases; it is uniformly low in the first case and uniformly high in the second.

Data for specific chromosome pairs are shown in Fig. 4.2(B)(i) and (ii) (for 18 and 19, blue and red respectively) as well as Fig. 4.2(B)(iv) and (v) (for chromosomes 12 and 20, blue and red respectively), corresponding to the activity distributions in
Fig. 4.2(A)(i) and Fig. 4.2(A)(ii) directly above them. Both show that chromosomes are distributed uniformly with respect to their gene density, with $S(R)$ showing the expected quadratic increase towards the nuclear periphery. The marginal differences between the distributions for the constant activity case reflects both interactions and the poly-dispersity of chromosomes; they also include the effects of entropic interactions between chromosomes. Such effects are overwhelmed once inhomogeneous activity, to be discussed next, is incorporated into the model.

Figure 4.2: (A)(i) – (iii) show a cut-away section illustrating the time-averaged local activity (see text). Data for the chromosome pairs 18 (red filled symbols) and 19 (blue filled symbols) ((B)(i) – (iii)), as well as for 12 (red filled symbols) and 20 (blue filled symbols) ((B)(iv) – (vi)), are shown corresponding to the activity distributions above them. Along with the simulation data in (B)(iii) and (B)(vi) (filled symbols), we also show open symbols in the same chromosome-specific colour representing the experimental data displayed in Ref. [36] for these chromosomes. Error bars indicated refer to standard deviations.

Fig. 4.2(A)(iii) summarizes a major result of our work: the non-uniform spatial distribution of local activity which follows when active temperatures, reflecting the
strength of local non-equilibrium force fluctuations, are assigned in a manner proportional to gene density. The distribution of activity differs strikingly from that shown in Fig. 4.2(A)(i) and (ii). Activity distributions in Fig. 4.2(A)(iii), reflecting gene densities, peak towards the centre and are suppressed towards the periphery, indicating gene-density-based segregation. In comparison to the case for uniform activity, (shown in Fig. 4.2(B)(i) and (iv) and Fig. 4.2(B)(ii) and (v) and discussed previously), Fig. 4.2(B)(iii) shows $S(R)$ for the inhomogeneous activity case. Experimental data for $S(R)$ associated to chromosomes 18 and 19 in human peripheral lymphocytes is also plotted (open symbols) for comparison. These figures, in distinct contrast to those of Fig. 4.2 (A)(i) – (ii), clarify that inhomogeneous activity drives the segregation of chromosomes, with the more gene-dense chromosomes found towards the centre of the nucleus. Our model, despite its simplicity, yields results which quantitatively reproduce the experimental magnitude of these relative shifts. $S(R)$ plots for all chromosomes are available in Fig. 4.3 for the model without loops but with non-equilibrium activity.

![Figure 4.3](image_url)

Figure 4.3: $S(R)$ for all chromosomes labelled 1, ..., 22 and X, computed for a spherical geometry and for inhomogeneous activity, illustrating how such distribution functions vary depending on the size and activity of the chromosome. Of these, we compare distributions for chromosomes 18 and 19 as well as 12 and 20 to experimental data.

Fig. 4.4(A)(i) – (ii) shows cut-away spheroids, both prolate as in Fig. 4.4(A)(i) as well
as oblate in Fig. 4.4(A)(i), exhibiting the time-averaged local activity. Fig. 4.4(B)(i) – (iv) showing $S(R)$ for the chromosome pairs 18 (red) and 19 (blue) as well as for chromosomes 12 (red) and 20 (blue), in the case of purely passive (geometrical) confinement, but accounting for inhomogeneous activity. These figures resemble those of Fig. 4.2(A)(iii) and Fig. 4.2(B)(iii) and (vi) both qualitatively and quantitatively. We also see no substantial difference in the relative positioning of chromosomes *vis a vis* the spherical case. This suggests that effects arising from purely geometric confinement are likely to be intrinsically small and that nuclear shape, acting on its own, may not be a strong determinant of chromosome positioning.

Figure 4.4: (A)(i) – (ii) show a cut-away section representing the time-averaged local activity (see text) for the case of a prolate ellipsoid in (A)(i) and an oblate ellipsoid in (A)(ii). Data for the chromosome pairs 18 (red filled symbols) and 19 (blue filled symbols) ((B)(i) – (ii)), as well as for 12 (red filled symbols) and 20 (blue filled symbols)((B)(iii) – (iv)) are shown. We also show the experimental data displayed in Ref. [36] as open symbols in the corresponding chromosome specific colours.
4.2 Modulation of chromosome positioning by the nuclear membrane

Genome organization is often tissue specific e.g. mouse chromosome 5 is more centrally positioned in liver cell nuclei but not in nuclei from lung tissue, where it is seen more peripherally [92]. Activity-based segregation favours the conventional arrangement, in which gene-poor heterochromatin is found towards the nuclear periphery, whereas gene-rich euchromatin regions are found towards the center of the nucleus. However, arrangements inverted with respect to the conventional one have been seen in rod cells from the retina of mice, most likely reflecting adaptation to a nocturnal lifestyle [24]. In these cells, all genes, irrespective of their transcriptional status, were found localised toward the nuclear periphery, in most cases juxtaposed to the nuclear lamina [24]. Recent experiments suggest that inversion in this case is a consequence of the absence of specific lamins and proteins (lamin A/C and lamin B receptor) which normally act to tether heterochromatin to the nuclear envelope [93]. Chromatin interactions with the nuclear lamina, en route to lineage commitment and terminal differentiation, have been suggested to play a central role in genome reorganisation [34,35]. Changes in these interactions may possibly limit the rate at which both cellular differentiation and reprogramming occur [94–96]. The approximations we have made preclude cell-type-specific analyses, but our model permits us to identify a fraction of monomers which can then interact preferentially with the nuclear envelope. We can then ask the following question: Can a suitably chosen fraction lead to stable inverted arrangements as terminal states? (We study this specific question because the conventional and inverted arrangement are two extreme limits of non-random radial distributions ordered by gene-density; if our model can recover these extremes, reproducing any radial positioning pattern intermediate between them should be straightforward). To model the selective interactions of monomers with the nuclear envelope, we make a simple approximation. We allow the wall to selectively interact with a fraction of the total number of monomers contained within our model nucleus, via a short-range attractive potential. To further simplify, we allow the nuclear envelope to act specifically on active monomers through a short-ranged attractive potential, with a minimum proximate to the nuclear envelope. Inactive monomers experience the usual repulsive confining potential. Thus, 5% of our monomers are both active as well as interact selectively with the nuclear envelope.

This attractive interaction is computed in the following way. Following the methodology described in Section 3.3, we calculate $\Delta R^2_i$, the closest (squared) distance between
4.2 Modulation of chromosome positioning by the nuclear membrane

Figure 4.5: (A)(i) – (iii) show a cut-away sphere, prolate ellipsoid and oblate ellipsoid displaying the time-averaged local activity (see text). (B)(i) shows the total density ($\rho(R)$) filled circles) and the gene density ($S^T(R)$), open circles) for the case of inhomogeneous activity but with a passive interaction with the nuclear envelope, while (B)(ii) shows these quantities in the presence of a selective interaction of active monomers with the inner surface of the simulated nucleus.

A monomer labelled by $i$ and the nuclear envelope. We choose the wall potential to be the following

$$V_{\text{wall}} = \frac{V_{\text{conf}}}{a^5} (\Delta R_i)^5 \quad \text{for } |\mathbf{r}_i| > R_0$$

$$= -V_{w0} \exp\left(-\frac{\Delta R_i^2}{2\sigma_w^2}\right) \quad \text{for } |\mathbf{r}_i| \leq R_0 \text{ and for all active monomers}$$

$$= 0 \quad \text{for } |\mathbf{r}_i| \leq R_0 \text{ and for all inactive monomers} \quad (4.1)$$

where $V_{w0} = 60 k_B T_{eq}$ and $\sigma_w^2 = 15.0$.

The choice of the depth of the potential well, as well as of its range, is to some extent arbitrary, since this procedure isolates the complexities of chromatin-nuclear envelope interaction into an extremely simplified form. However, since we expect that such an
interaction should be strong enough to be counter the natural tendency of chromosomes to segregate by (inhomogeneous) activity, the depth of this interaction should exceed the upper limit for active temperatures. The relative strength of this effect is then dictated by the Boltzmann factor \( \sim \exp \left( \frac{-60k_B T_{eq}}{20k_B T_{eq}} \right) = \exp (-3) \sim 0.05 \), strong enough to produce an appreciable wall effect, yet not so strong as to dominate completely over the intrinsic tendency towards activity-based segregation. The thickness of the lamins supporting the nuclear envelope is about 40-100 nm, but nuclear lamins also extend into the nucleoplasm and other nuclear envelope proteins add to this effective thickness; with the assumptions above, the length scale set by the wall interaction in our model is \( \sqrt{15 \times 28 \text{nm}} \approx 108.4 \text{nm} \).

The form the monomer-wall potential taken in this case is shown in Fig. 3.4(b).

Adding such an interaction dramatically alters positioning patterns. Fig. 4.5(A)(i) – (iii), show activity distributions corresponding to spherical, prolate and oblate nuclei, illustrating that activity now peaks in a narrow band towards the nuclear periphery, representing an increased density of genes in proximity to the nuclear envelope. Below these, we show, in Fig. 4.5(B)(i) – (ii), the total chromosome density (filled circles), as measured in our coarse-grained 1MB units, as a function of the radial coordinate \( R \), as well as the total gene density (open circles), for the case of inhomogeneous activity. There is no selective interaction with the nuclear envelope in the data shown in Fig. 4.5(B)(i) while the effects of such an active interaction is included in the results of Fig. 4.5(B)(ii). While both data show a quadratic rise close to the origin, the number of genes close to the nuclear envelope is substantially enhanced in Fig. 4.5(B)(ii).

Fig. 4.4 and Fig. 4.5 thus jointly illustrate the following: Purely geometrical effects arising from nuclear shape are weak, at least in our model. However, making the nuclear envelope selectively attractive to a small fraction of monomers can drastically alter positioning patterns, even inverting the conventional arrangement based on gene density. Thus, the competition between bulk (activity-based segregation) and surface (selective interactions of monomers with the nuclear envelope) e.g. see Ref. [93], provides a route to radial positioning schemes different from the conventional one, even though only a relatively small fraction of monomers - 5% in this case - selectively interacts with the nuclear envelope.

In our model, we can also investigate questions of more dynamical significance, such as the intermediate distributions obtained between two terminal ones, following the
initiation of a perturbation. We have investigated a specific perturbation, the reorganisation of chromosome positions in response to a nuclear envelope perturbation acting as described above, which leads to inverted arrangements as terminal steady states. In Fig. 4.6, we show the evolution of $S(R)$ as a sequence of snapshots of configurations of chromosomes 18 and 19, as tracked from the time-instant where the nuclear envelope interaction is switched on. We show, in sequence, through Fig. 4.6 (i) – (vi), the evolution of $S(R)$ captured in units of time-steps measured in millions of time-steps; Fig. 4.6 (vi) corresponds to the final steady state distribution. Initial configurations are drawn from a steady-state ensemble prepared by taking the nuclear confinement to be passive. This sequence illustrates how sequences intermediate between steady-state ones can be attained and possibly stabilised by other processes, not included in the present description, yielding a broad variety of distributions in $S(R)$. Within the assumptions we make, our model can thus provide predictions for distribution functions at intermediate times, arising out of a perturbation.

![Figure 4.6](image-url)

Figure 4.6: Evolution of the positional distribution of chromosomes 18 (red) and 19 (blue), shown as a function of time in sequence for (i) – (vi) as indicated, following the switching on of the active interaction with the nuclear envelope. Time is measured in units of millions of simulation time-steps as shown in each sub-graph and $\infty$ denotes the steady state.
4.3 Emergence of chromosome territories

Activity is a more potent driving force than entropy, since associated characteristic energy scales far exceed those set by physiological temperatures. One might guess that individually more compact configurations, however they might be generated, could be more effectively segregated by activity than by purely entropic means. Recall that in Fig. 4.1(D), we displayed a configurational snapshot of monomers on the nuclear surface, with each chromosome coloured differently, for the case of non-compact chromosomes with inhomogeneous activity. The relatively random distribution of colours on the surface indicates that chromosomes are intermingled. No visible tendency towards territory formation is seen. In Fig. 4.1(E), a similar snapshot is provided for the non-equilibrium case but initialised from an ensemble of more compact chromosome configurations, created via the random loop model. Remarkably, such configurational snapshots show consistent and strong evidence for a territorial organization, visually resembling the standard 3d-FISH images used to infer a territorial organization of chromosomes.

Figure 4.7: (A) shows instantaneous configurations of chromosomes 18, 19, 12 and 20 for the random loop model with inhomogeneous activity. In (B) the probability distribution of chromosomes 18 (red filled symbols) and 19 (blue filled symbols) in the compact case is shown and (c) shows the probability distributions for chromosomes with similar gene-densities, chromosomes 12 (red filled symbols) and 20 (blue filled symbols). Again, as before, the corresponding experimental data of Ref. [36] is shown as open symbols of the same colour. The agreement between experiment and simulation is close.

This is further examined in Fig. 4.7(A), where we show configurations of chromosomes 18, 19, 12 and 20 for the random loop model with inhomogeneous activity, providing
4.3 Emergence of chromosome territories

evidence for spatial separation and considerably reduced intermingling of chromosome segments in the bulk. The looping probability chosen is $3.0 \times 10^{-3}$. In Fig. 4.7(B), we display the probability distribution $S(R)$ for chromosomes 18 and 19 (blue and red, respectively) in the compact case, indicating that the activity-based segregation studied earlier is a phenomenon robust to allowing for such drastic changes to the configurational properties of individual chromosomes. As before, probability distributions for chromosomes with similar gene densities, as chromosomes 12 and 20 (blue and red, respectively in Fig. 4.7(C), show no segregation. Allowing for compactness substantially improves the agreement between simulations and experimental data, as can be seen by comparing Fig 4.2(B)(iii) and (vi) with Fig. 4.7(B) and (C).

To what extent is such territorial organization a robust feature of our model? To address this, we compare the final chromosome configurations which result from a number of separate random initialisations, to check that such a territorial organization is manifest across them. Fig. 4.8(A) shows four such configurations, obtained in steady state by simulating from independent initial states in which the monomers belonging to each chromosome are positioned at random. Each colour represents a separate chromosome. Note that each of these configurations separately shows a territorial organisation of chromosomes, although these configurations are not identical, illustrating that such organisation is a natural property of the steady state of this model. In particular, no fine tuning or specific choices of initial conditions is required for producing chromosome territory, as in some earlier work [36], but it arises naturally and in an emergent way from the twin requirements of activity-based segregation and compact chromosome configurations, in this case enforced through the random loop model for chromosomes. We note that recent polymer-based models for territoriality invoke the topological properties of ring polymers, assigning a crucial role to the inability of polymeric DNA to cross at intersections [97]. However, the relevance of such topological constraints is hard to assess since, in contrast to in vitro polymer systems for which such constraints are effectively absolute, DNA-topology-modulating enzymes such as type-II topoisomerases, specifically capable of relaxing such constraints, are present in large numbers within the nucleus.

To further quantify this visual result, we evaluate a quantity $Q(R_{sphere})$. In order to calculate this quantity, we construct a sphere of radius $R_{sphere}$ about the centre of each monomer, which we are free to vary. The sphere, if large enough, contains the centres of multiple monomers. For monomers belonging to the same chromosome as the initially selected monomer, we assign +1, while for monomers belonging to
4.3 Emergence of chromosome territories

Figure 4.8: (A) Four steady state surface configurations of monomers belonging to different chromosomes, colour coded by chromosome, with each configuration obtained using a different initialisation. (B) shows variation of $Q(R_{\text{sphere}})$ for three different values of the looping probability: 0, $1.5 \times 10^{-3}$ and $3.0 \times 10^{-3}$, yielding 424, 712 and 1404 loops (in one specific realisation) for the 6098 monomers.

For different chromosomes, we assign $-1$, summing these over all monomers whose centres are contained within the sphere. We then plot this summed quantity, which we call $Q(R_{\text{sphere}})$ averaged over all monomers belonging to all chromosomes. $Q(R_{\text{Sphere}})$ provides an indication of whether chromosomes are organized territorially and in a non-overlapping manner. Fig. 4.8(B) shows that for more compact chromosome configurations induced through a larger number of random loops, the tendency for overlap is reduced, further enhancing the tendency to separate which is a consequence of activity-induced segregation. Note that for large $R$, this quantity is negative, decreasing further as $R_{\text{sphere}}$ is increased. This reflects the fact that at large enough scales, the sphere drawn about each monomer contains multiple monomers belonging to different chromosomes. If there are no loops, substantial intermingling results, and this probe of territoriality remains negative, reflecting the presence of monomers belonging to other chromosomes in the near vicinity of a monomer belonging to a given one. This is the case for loop probability 0. The peak in $Q(R_{\text{sphere}})$ can tentatively be assigned to an averaged territory size, although the physical interpretation of this quantity is complicated by the fact that it is averaged over chromosomes of different sizes.
4.4 Size dependent segregation

So far we have been concentrating on $S(R)$, the monomer fraction at a distance $R$ from the centre of the confining sphere. We showed that inhomogeneous activity, confinement and the addition of random loops enables model polymers to mimic the structure and organisation of chromosomes in the eukaryotic cell nucleus. In this case, $S(R)$, represents DNA content as a function of the radial distance from the nuclear centre.

The behaviour of confined, active polymers is complex and their radial organisation depends on the quantity being observed. For example, instead of $S(R)$ we focus on the position of centre of mass of a chromosome, then our conclusion would be somewhat different. We illustrate below.

Kalhor et al. [10] showed using population based analysis [10] obtained from chromosome capture experiments (see Section 1.5.2) that the radial positions of chromosomes tend to increase with their size except for few chromosomes. These positions also strongly concur with the results of independent FISH experiment [10] in lymphoblasts. The radial positions are the positions of centre of mass of chromosomes. We check whether our model is capable of generating similar size dependent segregation. In order to do so we assigned temperatures $T_a = 12.0 \ T_{eq}$ to active monomers and $T_i = 6.0 \ T_{eq}$ to inactive monomers along with the random loop model with looping probability $3.0 \times 10^{-3}$, yielding approximately 1400 loops (in one specific realisation) for the 6098 monomers. Since we can assume that active fluctuations associated to the inactivated X-chromosome are negligible, we assign it an active temperature of 1 (thermal equilibrium), whereas all other chromosomes including the active X, are assigned an active temperature according to their gene density, including a background contribution, as discussed above. We then calculate centre of mass of each chromosome averaged over 10 initial configurations and 2.0 million time steps giving 0.4 million time steps to the system for getting steady state.

The center of mass of each chromosome is calculated using the relation below.

$$\vec{R}_{CM} = \frac{1}{N} \sum_{i=1}^{N} \vec{R}_i$$  \hspace{1cm} (4.2)

where $\vec{R}_i$ represents the position of $i^{th}$ monomer of a chromosome, $\vec{R}_{CM}$ is the centre of mass of that chromosome and $N$ is the number of monomers in the chromosome.
Our results are shown in Fig. 4.9, plotted together with the experimental results of Kalhor et al. It is clear that the two sets of data, theoretical and experimental lie within the range of uncertainties (error bars) of each other. Note that while the error bars for the experimental data has been taken from Ref. [10], our error bars are the standard deviations of the probability distributions of centre of mass of chromosomes.

There are a few comments that we need to make before we end this section. Firstly, note that our assignment of $T_a$ and $T_i$ is different here than what we have used before. The active temperature, therefore cannot be taken as an universal number but may vary with the cell type and the level of transcription. Secondly, we do not have data for $S(R)$ as well as centre of mass positions from the same experiment. Such data would be needed to validate our approach unequivocally. Lastly, in the next chapter we take up this problem again in the context of active polymers in confinement, abstracted away from its biological context. We show how different measures of segregation yield different results depending on activity.
4.5 Discussion

The higher-order organization of chromatin subtly modulates gene expression programs via epigenetics, linking local gene expression with the substantially larger physical scales of chromatin structuring and chromosome positioning [98–102]. Here, we showed how one form of such higher-order organisation, the robust radial segregation of chromosomes based on gene density, could be obtained within a relatively simple model. Previous work implicitly assumed chromosomes to be in thermal equilibrium. However, all available evidence indicates that stochastic forces associated to ATP-consuming (active) processes involved in chromatin remodelling and transcription dominate over thermal forces [77, 78]. Arguing that the inhomogeneous distribution of genes on each chromosome should lead to a similar inhomogeneity in the non-thermal (active) noise experienced by chromosomal segments with differing levels of active and inactive genes, we proposed that the relatively-recently understood physical phenomenon of segregation in systems of active particles with varying motility might underly gene-density-based chromosome segregation.

Assuming that gene density, once coarse-grained over a 1Mb region, could be taken as roughly representing the magnitude of transcription-linked activity within that region, we showed that segregation of the appropriate magnitude could be reproduced in a relatively simple model for interphase chromosomes. The predictions of this model include a detailed study of positioning of all human chromosomes, illustrating how chromosomes are differentially distributed as a function of their gene density. For the case of chromosomes 18 and 19 as well as of 12 and 20, our predictions compare favourably to experimental data on chromosome positioning in near-spherical human lymphocyte nuclei.

While purely geometrical effects appear weak a priori, the fact that the sphere is simply the geometrical figure with the smallest surface area at given volume suggests that surface specific interactions are likely enhanced in non-spherical geometries, such as in the fairly flat cells in which size-dependent segregation was seen earlier. Adding a selective interaction drawing active monomers towards the nuclear envelope altered positioning, stabilising distributions inverted with respect to the conventional one. Thus, we demonstrated that two extreme limits of gene-density-dependent radial distributions could be generated, stably and reproducibly, with minimal assumptions.

We found that combining mechanisms ensuring more compact chromosomes with the intrinsic, activity-derived tendency of chromosomes to segregate led directly to
configurations closely resembling the territorial organisation seen in the FISH imaging of individually painted chromosomes. This striking result, obtained without any requirement for fine-tuning, leads us to suggest that non-equilibrium activity may be largely responsible for the territorial organisation of chromosomes. Allowing for the formation of a small number of loops, thus generating more compact configurations of individual chromosomes, maintained activity-based segregation, while also yielding closer agreement with the experimental $S(R)$.

A central requirement of our model is that local measures of the magnitude of stochastic non-equilibrium forces experienced by different sections of individual chromosomes should correlate to local ATP-consuming enzymatic activity. When gene-density-based segregation is initially established, likely in early G1 where chromosome movements are largest [103], we expect that the scale of such stochastic forces should decrease in a quantifiable manner between nuclear centre and periphery, as Fig. 4.2(A)(iii) indicates. DNA-repair machinery is ATP-consuming, and we consider it plausible that the reversible chromosome territory movements observed during DNA damage repair reflect varying levels of chromosome-specific internal repair-related activity [104]. Correlating chromosome-specific levels of damage to alterations in positions should test our hypothesis that the scale of active forces experienced by individual chromosomes is related to the positions they occupy relative to the nuclear centre. In addition to the situations listed above, chromatin remodelling performs a central epigenetic regulatory function in replication, apoptosis and development, suggesting that at least some fraction of large-scale chromosome movements in these situations may reflect inhomogeneous activity of the sort we discuss here. Finally, in vitro studies of positioning patterns involving artificial chromosomes, where activity can be induced or suppressed in a controlled manner, may provide tests of our hypothesis that activity controls large-scale positioning.

4.6 Conclusion

Radial gene-density-based chromosome organisation and territory formation may represent foundational principles of spatial genome structuring which are common to multiple cell types [7]. The mechanism we propose for these is attractive because it is general and robust. Our work also clarifies why previous attempts to understand segregation from purely equilibrium considerations were incomplete. Given the generality of these ideas, we expect that they may be applicable to large-scale DNA
organisation in lesser eukaryotes as well as in bacteria [105].

In Meaburn and Misteli’s “self-organisation model” for chromosome positioning, “the position of each chromosome is largely determined by the activity of all its genes; that is, the number and pattern of active and silent genes on a given chromosomes” [6]. Here, we link such patterns of gene activity directly to physical positioning via inhomogeneous activity, suggesting a specific physical mechanism for such self-organisation.

The importance of non-equilibrium activity for theoretical descriptions of chromatin and the demonstration that gene-density-based segregation and the formation of territories have a common and robust underlying origin in our model system via “activity-based segregation”, are the central results of our work. We suggest that activity-based segregation might provide a generic initial template for local physical and biochemical events acting to further stabilize and optimize positioning. These include, but are not limited to, the effects of nuclear shape at larger aspect ratios, selective interactions with the nuclear envelope and associated chromatin-binding proteins, potential transient interactions with localised dynamic clusters of nuclear myosin motors and actin, as well as, importantly, the spatial clustering of active genomic regions associated to the intermingling of looped chromosomal segments in inter-chromatin domains or at transcription factories [5, 106]. Each of these would supplement such a generic template with incremental layers of cell-type-specific organisation. Generalising our model to incorporate these effects should yield more complex proximity patterns. Comparing such in silico predictions to experimental data at increasing levels of microscopic resolution can be expected to yield useful insights into the multiple determinants of chromosome positioning.