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Macrogenomic engineering via modulation of the scaling of chromatin packing density

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Table of Contents

1.Macrogenomics Model and Analysis

1.1 Chromatin-Packing Macromolecular-Crowding Model

The average expression rate of a group of genes, E, with similar molecular characteristics, $\vec{m}$, and gene length, L, can be approximated as:

(1) $E = ASA \cdot \bar{\epsilon}$

where ASA is the probability of a gene being on an accessible surface and $\bar{\epsilon}$ is the mRNA rate of expression of the accessible genes.

To integrate these effects into a unified model, the chromatin-packing macromolecular-crowding (CPMC) model, we consider the fact that the fractal nature of chromatin governs both the overall accessible area as well as the distribution of mass density. The morphology of a fractal can be characterized by the fractal dimension D, which is defined by[1]:

(2) $P \propto s^{D-3-d}$

where s is the scale, P is the value of the measured property at scale s, and d is the topological dimension ($d=3$ for mass and $d=2$ for surface area). This produces the following relationships for the fractal:

(3) $\frac{M_f}{M_{min}} = \left(\frac{r_f}{r_{min}}\right)^D$

(4) $\frac{S_f}{S_{min}} = \left(\frac{r_f}{r_{min}}\right)^{D-1}$

where M_f, M_{min}, S_f, S_{min}, r_f and r_{min} are the mass of the fractal, the mass of the elementary structure of the fractal, the accessible surface area of the fractal, the accessible surface area of the elementary structure of the fractal, the radius of fractal, and the radius of the elementary structure in the fractal respectively. In chromatin, M_f, S_f, and r_f are the mass, accessible surface area, and radius of the region of a chromosome for which a power-law scaling holds, and M_{min}, S_{min}, and r_{min} are the mass, accessible surface area, and radius of a single double stranded DNA basepair (r_{min} ~1nm). Therefore, the expectation of the fraction of chromatin that corresponds to accessible surface area (ASA) can be approximated as:

(5) $ASA = \left(\frac{S_f}{S_{min}}\right) / \left(\frac{M_f}{M_{min}}\right) = \left(\frac{M_f}{M_{min}}\right)^{-1/D}$

We note that ASA increases with D if M_f is conserved.

The average mRNA expression rate $\bar{\epsilon}$ depends on the mRNA expression rate at constant chromatin mass density and the distribution of DNA and DNA associated proteins (i.e. chromatin), the second of which can be considered as the predominant macromolecular crowder within the nucleus:

(6) $\bar{\epsilon} = \int \epsilon(\vec{m}, \phi) f(\phi) \mathrm{d}\phi$

where $\epsilon(\vec{m}, \phi)$ is the rate of expression of genes as a function of the set of molecular regulatory features $\vec{m}$ and the average crowding density within the transcriptional interaction volume ϕ, and $f(\phi)$ is the probability distribution function (p.d.f.) of the crowding densities to which the genes within the gene group can be exposed. The transcriptional interaction volume is the space within which transcriptional reactions are influenced by the local macromolecular density. The radius L_{in} of this volume is

determined by the distance at which macromolecular crowding no longer influences the binding affinity of polymerases and transcription-factors during chemical reactions. Notably, the size of the interaction volume is also dependent on the size of gene L. Considering the fractal nature of chromatin, the relation between L_{in} and L can be approximated as:

$$(7)\ L_{in} = L_{in}^{0} + L^{1/D} r_{min}$$

where L_{in}^{0} is the radius of the interaction volume for a single base pair of a gene and is approximated as 15 nm based on the Monte Carlo simulation of crowding effect[2]. Previously, the study of the effect of macromolecular crowding on transcription showed a non-monotonic dependence of $\epsilon(\vec{m}, \phi)$ on ϕ.[2] In relation to chemical reactions (i.e. transcription), macromolecular crowding has a two-fold effect: (1) it decreases the mobility (diffusion) of the reactant species involved in forming transcriptional complexes, and (2) it increases the binding affinity of the reactant species due to excluded volume interactions. The fractal nature of chromatin determines the local mass density distribution of macromolecular crowders. The primary structures of chromatin are packed into clusters along a hierarchy of length-scales, and these clusters are considered to be the main crowders in the nucleus. The fractal dimension of chromatin determines the size distribution of these crowders, and $\bar{\epsilon}$ can be approximately evaluated from Eq.6 by expanding $\epsilon(\vec{m}, \phi)$ into the Taylor series and then integrating each of the terms:

$$(8)\ \bar{\epsilon} \approx \int \left[\epsilon(\vec{m}, \bar{\phi}) + \frac{\partial \epsilon(\vec{m},\phi)}{\partial \phi}\Big|_{\bar{\phi}} (\phi - \bar{\phi}) + \frac{1}{2} \frac{\partial \epsilon^2(\vec{m},\phi)}{\partial \phi^2}\Big|_{\bar{\phi}} (\phi - \bar{\phi})^2 \right] f(\phi) d\phi$$
$$= \epsilon(\vec{m}, \bar{\phi}) + \frac{1}{2} \sigma_{\phi_{in}}^2 \frac{\partial \epsilon^2(\vec{m}, \phi)}{\partial \phi^2}\Big|_{\bar{\phi}}$$

where $\bar{\phi}$ is the average crowding density in the nucleus and $\sigma_{\phi_{in}}^2$ is the variance of the average intra-interaction volume density across multiple interaction volumes. $\sigma_{\phi_{in}}^2$ can be estimated by considering the power-law scaling property of chromatin.

$$(9)\ \sigma_{\phi_{in}}^2 = B(\vec{r} = 0) = \int B_c(\vec{r}) ACF_{in}(\vec{r}) d\vec{r} \approx \bar{\phi}(1 - \bar{\phi}) ({}^{r_{min}}/_{L_{in}})^{3-D}$$

where $B(\vec{r})$ is the auto-correlation function of ϕ, $B_c(\vec{r})$ is the auto-correlation function of crowding density at each point within the nucleus, and $ACF_{in}(\vec{r})$ is the auto-correlation function for the shape function of the interaction volume $SH(\vec{r})$. Eq. (9) holds independent of the assumption of $SH(\vec{r})$ (e.g. Gaussian function or binary function). The first term in Eq. (8) describes the effect of macromolecular crowding on transcription in the absence of chromatin packing-density variations and the second term in Eq. (8) reveals the impact of the variations of chromatin packing density, which are in turn due to the power-law scaling of chromatin packing density. Here, we assume that $\bar{\phi}$ is within the physiological range (30-45%). Earlier Monte Carlo and Brownian Dynamics simulations predicted that this range of $\bar{\phi}$ maximizes $\epsilon(\vec{m}, \phi)$. In other words, on average the packing density of chromatin is near the optimal configuration. Since the effects of macromolecular crowding on ϵ cannot be experimentally obtained *in vitro*, $\frac{\partial \epsilon^2(\vec{m},\phi)}{\partial \phi^2}\Big|_{\bar{\phi}}$ in Eq.8 was estimated from the Monte Carlo and Brownian Dynamics simulations *in silico*[2]. Thus, the integration of the fractal nature of chromatin and the macromolecular crowding model gives us the estimation of the expression rate for mRNA.

In order to evaluate the behavior of chromatin in non-fractal conditions, we utilized the Whittle-Matérn (WM) functional family to model the auto-correlation function (*ACF*) of chromatin. The WM functions are a convenient and versatile choice as they cover a broad range of functional forms, including: fractal ($D < 3$), stretched exponential ($D \in (3,4)$), exponential ($D=4$), and Gaussian ($D \to \infty$).[3,4] In order to estimate how the total surface area of chromatin changes with *D,* we modeled the media as a combination of particles with a size distribution that would match the shape of the *ACF*. The probability density function of the size distribution of these particles can be approximated as:

$$(10)\ N(s,D,lc) = \frac{1}{s^2}\int ACF(s,D,lc)ds,$$

where *s* is the particle size and the WM *ACF* is given by

$$(11)\ ACF(r,D,lc) \propto \frac{2^{\left(\frac{5-D}{2}\right)} * \left(\frac{r}{lc}\right)^{\left(\frac{D-3}{2}\right)} K_{\left(\frac{D-3}{2}\right)}\left(\frac{r}{lc}\right)}{\left|\Gamma\left(\frac{D-3}{2}\right)\right|},$$

where lc is the length-scale of the *ACF* and *K* is the Bessel function of the second kind and $\Gamma()$ is the gamma function. The physical meaning of lc depends on *D*: for the fractal regime ($D<3$), lc is proportional to the upper length-scale of self-similarity, whereas for $D=4$, it is the 1/e correlation distance.

Using this probability density function to characterize the distribution of mass density, the probability of access to the genes and the local variation in mass density can be analytically calculated as a function of the WM *ACF* as:

$$(12)\ ASA = A\int_0^{R_{max}} N(s,D,lc) * s^2 ds,$$

$$(13)\ \sigma^2_{\phi_{in}} = \int_0^{R_{max}} ACF(\vec{r},D,lc) * ACF_{in}(\vec{r})d\vec{r},$$

where *A=const* depends on the shape of the particles and a fixed total mass of chromatin *M* and R_{max} is the maximum particle size. Owing to the fact that the total mass of a chromatin region can be calculated by

$$(14)\ M = \rho B\int_0^{R_{max}} N(s,D,lc) * s^3 ds \approx \frac{1}{2}\rho B\alpha(D) lc R^2_{max},$$

where ρ is the internal density of the particles, constant *B* depends on the particle shape, and $\alpha(D) = \frac{1}{lc}\lim_{s\to\infty}(N(s,D,lc) * s^2)$, we can approximate $R_{max} \sim \sqrt{2 * {}^{M}/_{\rho B\alpha(D) * lc}}$. By substituting R_{max} into equations (12) and (13), we can estimate how *ASA* and $\sigma^2_{\phi_{in}}$ depend on *D*. We find that both the accessible surface area and the local variations in mass density increase in non-fractal ($D>3$) conditions (**Supplementary Figure 4**).

In summation, the CPMC model predicts the effects of the scaling of chromatin packing density on the rate of gene expression by considering the inseparable tension between increased accessible surface area and variations in local crowding that depend on *D*. On one hand, as accessible surface area increases so too would the number of available binding sites throughout the genome. Thus, increased accessibility has a universally enhancive effect on transcription. The effective contribution from increased variations in local crowding are more nuanced and depend on the interplay between diffusivity and the stabilization of transcriptional complexes. As a function of crowding, transcription is a non-monotonic function that depends intrinsically on the initial expression state of a gene

and peaks within the physiological range of 30-45%[2]. This non-monotonic dependence of mRNA synthesis on molecular crowding is due to the 'competition' between two key physical effects that influence the probability of expression of a gene: molecular diffusion (e.g. transcriptional factors and other molecules that eventually form a transcription complex), which is suppressed by crowding, and the binding constants of formation of transcription complexes, which is enhanced by crowding. Under-expressed genes are far more sensitive to changes in crowding than over-expressed genes. The formation of the transcription complexes for these genes is the rate limiting step, and the increased binding probability due to crowding increases the probability of mRNA synthesis for these genes significantly more so than for already highly expressed genes with optimized binding probabilities. Consequently, the net effect of an increase in D is the gene expression enhancement for the majority of genes due to the greater surface area of chromatin and the concomitant suppression due to the greater variations in chromatin packing density within the interaction volume with the latter effect being more pronounced for the already under-expressed genes. In other words, this is a further enhancement of active genes and further suppression of partially suppressed genes.

1.2. Sensitivity

We analyze the sensitivity, *Se*, the response of a group of genes with different initial molecular and physical conditions to alterations in chromatin packing-density scaling as:

$$(15)\ Se = \frac{\partial \ln(E)}{\partial \ln(D)}.$$

Since the average expression rate for genes with similar characteristics is $E = ASA \cdot \bar{\epsilon}$, *Se* can be evaluated as:

$$(16)\ Se = D\left(\frac{\partial \bar{\epsilon}\,/\bar{\epsilon}}{\partial D} + \frac{\partial ASA/ASA}{\partial D}\right).$$

Substituting equations (5) and (8) into equation (16), we obtained:

$$(17)\ Se = \left[1 - {}^{1}\!/_{g(\bar{\epsilon},D)}\right] \cdot \left[D \ln\left(\frac{L_{in}}{r_{min}}\right) + \frac{3-D}{D}\frac{r_{min}}{L_{in}} L^{1/D} \ln(L)\right] + \frac{1}{D}\ln\left(\frac{M_f}{M_{min}}\right)$$

where the function $g(.)$ is the variable transformation function defined as:

$$(18)\ \epsilon(\vec{m},\bar{\phi}) \approx \bar{\epsilon} - \frac{1}{2}\sigma^2_{\phi_{in}} \left.\frac{\partial \epsilon^2(\vec{m},\phi)}{\partial \phi^2}\right|_{\bar{\phi}} = \bar{\epsilon}/g(\bar{\epsilon},D).$$

The Monte Carlo and Brownian Dynamics simulations of the effect of macromolecular crowding on gene transcription show that the relation between $\bar{\epsilon}$ and $\left.\frac{\partial \epsilon^2(\vec{m},\phi)}{\partial \phi^2}\right|_{\bar{\phi}}$ can be approximated as (**Figure 3B** in the main text)[2]:

$$(19)\ \frac{\left.\frac{\partial \epsilon^2(\vec{m},\phi)}{\partial \phi^2}\right|_{\bar{\phi}}}{\epsilon(\vec{m},\bar{\phi})} \approx -\sqrt{\frac{\kappa}{\epsilon(\vec{m},\bar{\phi})}}.$$

Therefore this function $g(.)$ can be analytically approximated as:

$$(20)\ g(\bar{\epsilon},D) = \frac{1}{1+\frac{1\kappa}{8\bar{\epsilon}}(\sigma^2_{\phi_{in}})^2\left(1+\sqrt{1+\frac{16\ \ \bar{\epsilon}}{(\sigma^2_{\phi_{in}})^2\kappa}}\right)},$$

where $\kappa = 22.6$ nM/s, is the critical rate of expression such that for $\bar{\epsilon} < \kappa$ crowding has a significant effect. Because κ exceeds the physiological range of the rate of transcription, crowding is expected to have a significant effect in gene transcription.

1.3. Intercellular Heterogeneity

Intercellular transcriptional heterogeneity (*H*) is the standard deviation of the transcription rate of a gene across a population of cells with the same *D*. The cell population-average transcription for a gene can be written as:

$$(21)\ E_s = ASA \cdot \bar{\epsilon}_s$$

where $\bar{\epsilon}_s$ is the population-average transcription rate of the gene that is accessible for transcription,

$$(22)\ \bar{\epsilon}_s = \int \epsilon\,(\vec{m}, \phi_s) f(\phi_s) \mathrm{d}\phi_s$$

where $\epsilon(\vec{m}, \phi_s)$ is the expression rate of the gene as a function of the interaction volume-averaged crowding ϕ_s, and $f(\phi_s)$ is the probability distribution function of ϕ_s across different cells within the cell population. The variance of ϵ_s, Var_{ϵ_s}, is then:

$$(23)\ Var_{\epsilon_s} \approx \frac{1}{4}\epsilon^{''}(\vec{m}, \overline{\phi_s})^2 (E[(\phi_s - \overline{\phi_s})^4] - E[(\phi_s - \overline{\phi_s})^2]^2)$$

where $\overline{\phi_s}$ is the mean of ϕ_s, $E[(\phi_s - \overline{\phi_s})^4]$ is the expectation of $(\phi_s - \overline{\phi_s})^4$, $\frac{\partial \epsilon(\vec{m}, \phi_s)}{\partial \phi_s}\Big|_{\overline{\phi_s}} = 0$, and, to simplify notations, $\epsilon^{''}(\vec{m}, \overline{\phi_s}) \equiv \frac{\partial \epsilon^2(\vec{m}, \phi_s)}{\partial {\phi_s}^2}\Big|_{\overline{\phi_s}}$. If f is a normal distributed, $E[(\phi_s - \overline{\phi_s})^4] = 3E[(\phi_s - \overline{\phi_s})^2]^2$. Thus:

$$(24)\ Var_{\epsilon_s} \approx \frac{1}{2}\epsilon^{''}(\vec{m}, \overline{\phi_s})^2 {\sigma_{\phi_{in,s}}}^4$$

where $\sigma_{\phi_{in,s}}$ is the standard deviation of ϕ_s across the cell population Therefore, the intercellular transcriptional heterogeneity can then be written as:

$$(25)\ H(D) = Var_{E_s}^{\frac{1}{2}} \approx \frac{1}{\sqrt{2}} ASA * \sigma^2_{\phi_{in,s}} \left|\epsilon^{''}(\vec{m}, \overline{\phi_s})\right| \approx \frac{1}{\sqrt{2}} ASA * \sigma^2_{\phi_{in}} \left|\epsilon^{''}(\vec{m}, \overline{\phi_s})\right|,$$

where Var_{E_s} is the variance of E_s.

Since *H* is difficult to measure experimentally, from a practical standpoint, there are two metrics of interest that can be derived from *H*. The first metric is the relative intercellular transcriptional heterogeneity between two cell states or populations with fractal dimensions D_1 and D_2, respectively:

$$(26)\ \frac{H(D_2)}{H(D_1)} = \frac{\sigma^2_{\phi_{in,s}}(D_2) ASA(D_2)}{\sigma^2_{\phi_{in,s}}(D_1) ASA(D_1)} = \left(\frac{M_f}{M_{min}}\right)^{-\frac{1}{D_2}+\frac{1}{D_1}} \left(\frac{r_{min}}{L_{in}}\right)^{-D_2+D_1}.$$

The second metric is the coefficient of variation of intercellular transcriptional heterogeneity, CV_E. CV_E can be found using the Taylor expansion of E_s:

$$(27)\ CV_E = \frac{Var_E^{\frac{1}{2}}}{E_s} \approx \frac{1}{\sqrt{2}} \cdot \frac{\sigma^2_{\phi_{in,s}} \left|\epsilon^{''}(\vec{m}, \overline{\phi_s})\right|}{\epsilon_s(\vec{m}, \overline{\phi_s}) + \frac{1}{2}\sigma^2_{\phi_{in,s}} \epsilon^{''}(\vec{m}, \overline{\phi_s})},$$

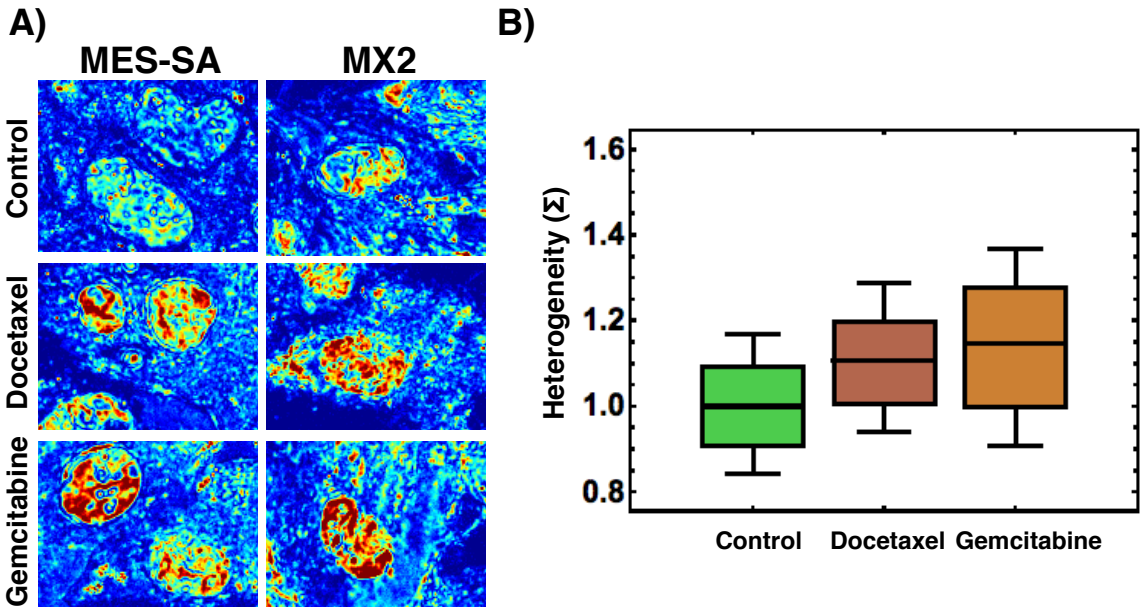
and substituting Eq. (18) into (27), CV_E is simplified:

$$(28)\ CV_E \approx \sqrt{2}\left(1 - \frac{1}{g(\bar{\epsilon}_{s,D})}\right) \cdot sgn\left[\epsilon^{''}(\vec{m}, \overline{\phi_s})\right] = \sqrt{2}\left(\frac{1}{g(\bar{\epsilon}_{s,D})} - 1\right)$$

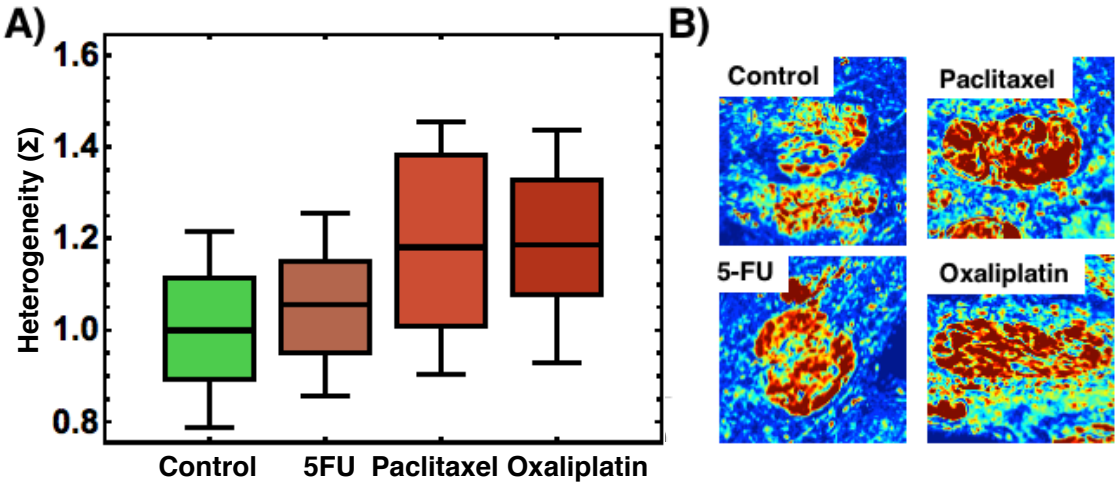
where $sgn\left[\epsilon^{''}(\vec{m}, \overline{\phi_s})\right]$ is the sign of $\epsilon^{''}(\vec{m}, \overline{\phi_s})$ and can be treated as -1. Since $\sigma^2_{\phi_{in}}$ increases with *D* (**Supplementary Figure 4B**) and *g(.)* decreases with $\sigma^2_{\phi_{in}}$ (Eq. 20), the

increase of D leads to the increase of CV_E. This trend was confirmed experimentally. (**Supplementary Figure 5**).

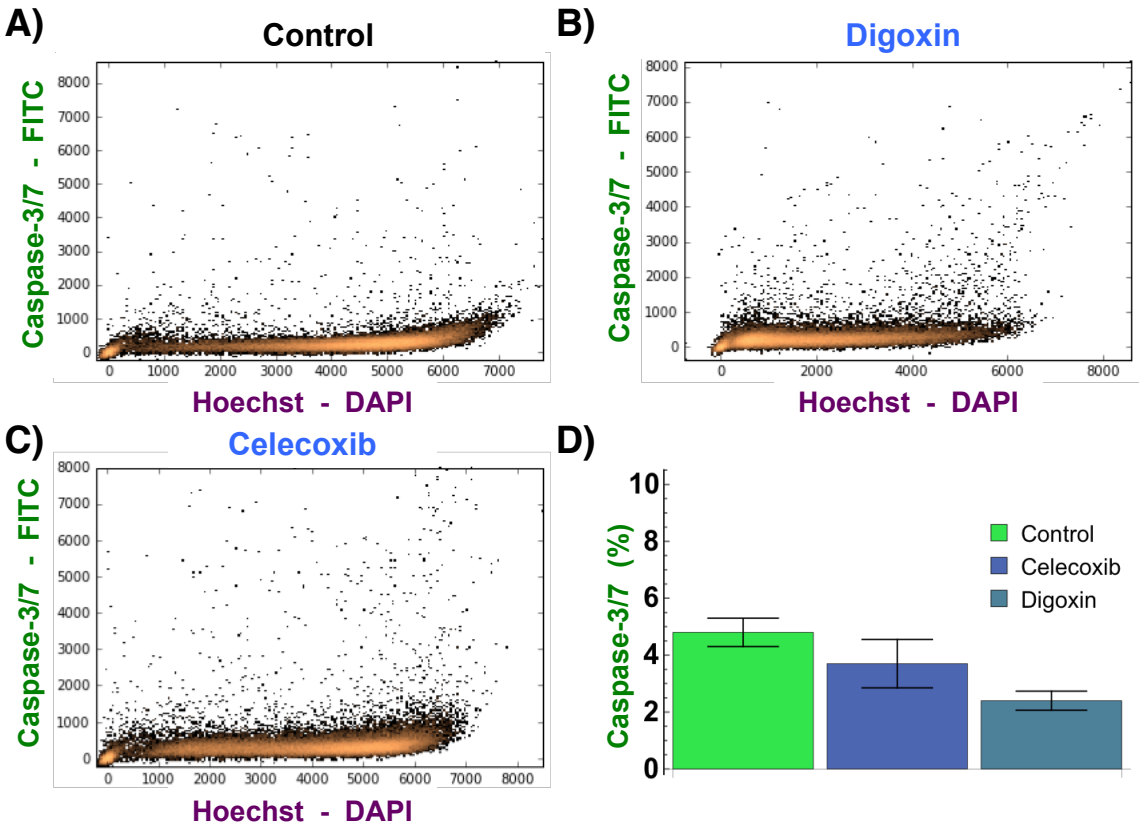
2. Supplementary Figures



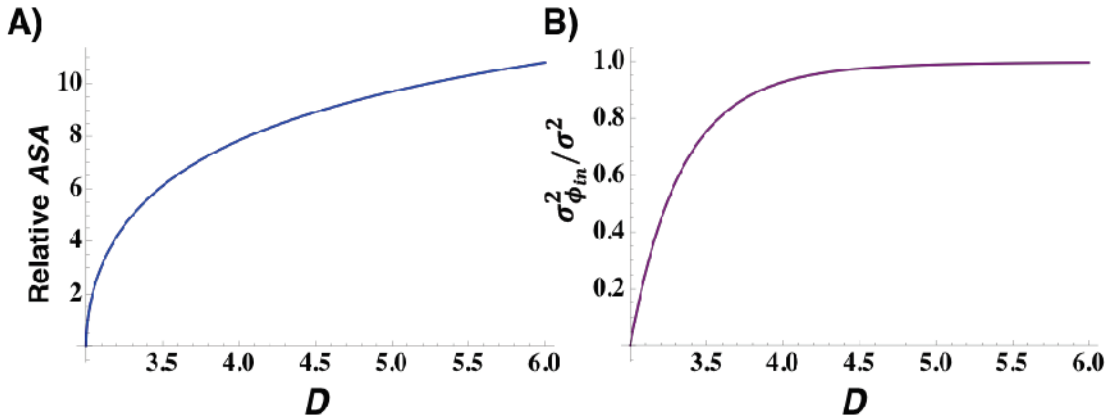
SI Fig. 1) Leiomyosarcoma cancer-cells treated with chemotherapy. **A)** Representative images of chromatin packing-density heterogeneity of the leiomyosarcoma MES-SA and mitoxantrone resistant derivative MESSA.MX2 (MX2) cells treated with gemcitabine and docetaxel for 48 hours. **B)** Chromatin heterogeneity is increased in MX2 derivative cells exposed to docetaxel ($p=6.6\times10^{-12}$) or gemcitabine ($p=3.6\times10^{-13}$) for 48 hours. Significance was determined using Student's t-test with unpaired, unequal variance on the average nuclear Σ normalized by the average Σ of the accompanying control group between the conditions. Box represents the 25-75% range and whisker represents the 10-90% range of values around the mean for N=558 control, 106 docetaxel treated, and 103 gemcitabine treated MX2 cells.



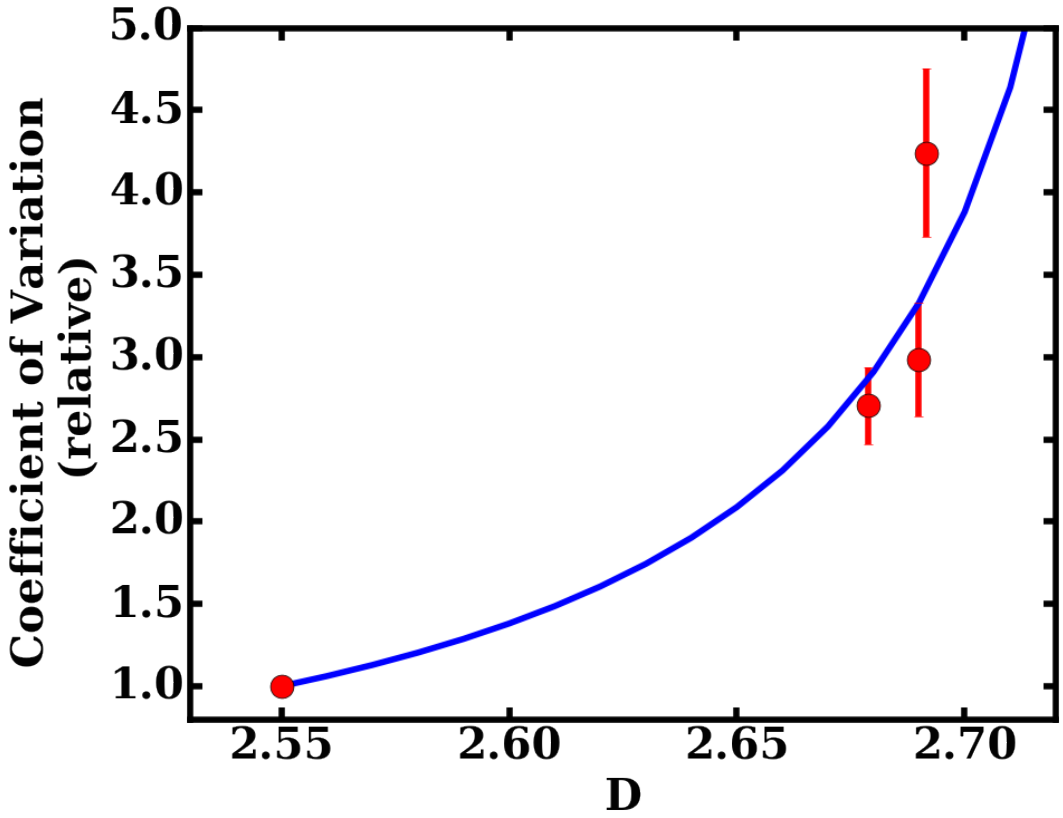
SI Fig. 2) Ovarian A2780.M248 (M248) cancer-cells treated with chemotherapy. **A)** Chromatin packing-density heterogeneity is increased in M248 derivative cells treated for 72 hours with 5-FU ($p=2.2\times10^{-3}$), or 48 hours with paclitaxel ($p=6.6\times10^{-7}$) or oxaliplatin ($p=5.0\times10^{-15}$). Significance was determined using Student's t-test with unpaired, unequal variance on the average nuclear Σ normalized by the average Σ of the accompanying control group between the conditions. Box represents the 25-75% range and whisker represents the 10-90% range of values around the mean for N=525 control, 100 5-FU treated, 45 paclitaxel treated, and 85 oxaliplatin treated A2780 cells. **B)** Representative images of chromatin heterogeneity of the ovarian carcinoma M248 cells treated with 5-FU, paclitaxel, or oxaliplatin for 48 hours.



SI Fig. 3) Monotreatment with chromatin protective therapies does not induce Caspase-3/7 activation. **(A-C)** Representative flow cytometry data of (**A**) control untreated, (**B**) 48 hour Digoxin treated, and **(C)** 48 hour Celecoxib treated A2780 cells. **D)** Quantification of the percentage of Caspase (Cas)-3/7 positive cells comparing untreated controls to 48 hour celecoxib or 48 hour digoxin treated cells (p=n.s., n=2). Error bars represent uncertainty based on ±10% change in gating thresholds.



SI Fig. 4) Extension of the model in non-fractal conditions. **A**) Fold change in accessible surface area in non-fractal conditions (*D*>3). *ASA* was scaled relative to the *ASA* for *D*~3. **B**) Variance of the interaction volume-averaged chromatin packing density across interaction volumes ($\sigma^2{}_{\phi_{in}}$) in non-fractal (*D*>3) conditions. $\sigma^2{}_{\phi_{in}}$ is normalized by the variance of chromatin crowding density at each point in the nucleus, σ^2. Even in non-fractal conditions, both the *ASA* and the local variations in density increase monotonically as a function of *D*.



SI Fig. 5) Coefficient of variation of intercellular gene expression. Model-predicted coefficient of variation for intercellular gene expression as a function of *D* (blue curve) and the coefficient of variation for intercellular gene expression calculated based on the experimental microarray data (red dots). The result is normalized by the coefficient of variation of cells with the lowest *D*. Error bars are the standard error of the coefficient of variation.

3. Supplementary References

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