Following DNA Compaction During the Cell Cycle by X-Ray Nano-Diffraction

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Supporting Information for Publication

Additional Figures

To support the results presented in the main article, additional figures are shown in this supporting document. Fig. S1 shows the Porod fits of the average ROI signal from six cells in analogy to Fig. 2a in the main text. Fig. S2 shows another example in analogy to Fig. 4 in the main text, with the dark-field, Porod exponent, Porod constant and surface-to-volume-ratio maps, together with the distribution of α and S with their respective fits. The distributions shown in Fig. 4 of the main text are fitted by Gaussian functions and the fits are provided in Fig. S3.

Surface-to-volume ratio $S_{\mathbf{v}}$

In Fig. 5c of the main text, the surface-to-volume ratio of three systems A, B and C, composed of "cubes" are compared. Tab. S1 provides the corresponding calculation of these three systems. We find that $S_v^{\rm C} > S_v^{\rm A} > S_v^{\rm B}$.

Table S1: Calculation of S_v for three different systems as represented in Fig. 5c of the main text. Case A is composed of 12 cubes of side length 2 (a.u.). Case B is also composed of 12 cubes of side length 2 (a.u.) but some are in contact. Case C is composed of 12 cubes of side length 0.5 (a.u.).

System	Face surface $(a.u.^2)$	Surface area $(a.u.^2)$	Volume $(a.u.^3)$	$S_{\rm v}~({\rm a.u.}^{-1})$
А	$2^2 = 4$	$6 \times 4 = 24$	$2^3 = 8$	$\frac{24}{8} = 3$
В	$2^2 = 4$	$4 \times 3 + 2 \times 1 = 14$	$2^3 = 8$	$\frac{14}{8} = 1.75$
С	$0.5^2 = 0.25$	$6 \times 0.25 = 1.5$	$0.5^3 = 0.125$	$\frac{1.5}{0.125} = 12$

Porod invariant calculation

In the main text, the Porod invariant Q is used to compute a map of the surface-to-volume ratio $S_{\rm v}$. The Porod invariant is:

$$Q = \int_0^\infty I(q)q^2 \mathrm{d}q.$$
 (S1)

As the scattering signal is not acquired from 0 to ∞ , this equation is separated into three terms:

$$Q = \int_{0}^{q_{\min}} I(q)q^{2} dq + \int_{q_{\min}}^{q_{\max}} I(q)q^{2} dq + \int_{q_{\max}}^{\infty} I(q)q^{2} dq,$$

$$Q = Q_{\text{Guinier}} + Q_{\text{Experimental}} + Q_{\text{Porod}}.$$
(S2)

The first term of this equation Q_{Guinier} can be estimated using the Guinier's approximation:

$$I(q) = I_0 \exp\left(-\frac{q^2 R_g^2}{3}\right) \tag{S3}$$

By substituting Eq. S3 into Eq. S2 we obtain:

$$Q_{\text{Guinier}} = \int_0^{q_{\text{min}}} I_0 \exp(-\frac{q^2 R_g^2}{3}) q^2 \mathrm{d}q.$$
 (S4)

Using a Taylor series expression of $\exp(-q) = 1 - q + \frac{q^2}{2!} - \frac{q^3}{6!} + \dots + \mathcal{O}(q^n)$ in Eq. S4, we obtain:

$$Q_{\text{Guinier}} = I_0 \int_0^{q_{\text{min}}} q^2 - \frac{q^2 R_{\text{g}}^2}{3} q^2 + o(q^3) dq,$$

$$Q_{\text{Guinier}} = I_0 \frac{q_{\text{min}}^3}{3} - I_0 \frac{q_{\text{min}}^5 R_{\text{g}}^2}{15},$$
since the Guinier approximation is only valid for $qR_{\text{g}} \ll 1$, so $q^5 \ll q^3$

$$Q_{\text{Guinier}} = I_0 \frac{q_{\text{min}}^3}{3}.$$
(S5)

The third term of Eq. S2 can be reduced using Porod's law $I(q) = Kq^{-4} + B$:

$$Q_{\text{Porod}} = \int_{q_{\text{max}}}^{\infty} I(q)q^2 dq,$$

$$Q_{\text{Porod}} = \int_{q_{\text{max}}}^{\infty} (Kq^{-4} + B)q^2 dq,$$

$$B \text{ is very small and can be neglected, thus} \qquad (S6)$$

$$Q_{\text{Porod}} = \int_{q_{\text{max}}}^{\infty} Kq^{-2} dq,$$

$$Q_{\text{Porod}} = \frac{K}{q_{\text{max}}}.$$

Beaucage model

As explained in the main text, the Beaucage model is used to unify the low and high q-values.^{1,2} The Beaucage model is very suitable for polymer fractal systems that show a non-integer Porod exponent and it provides the radius of gyration $R_{\rm g}$ and Porod exponent α . A smooth transition between the two q-regions (Guinier and Porod) is provided by an

error function. The scattering signal is modeled using three fit parameters G, $R_{\rm g}$, and α :

$$I(q) = G \exp\left(-\frac{q^2 R_{\rm g}^2}{3}\right) + \frac{C}{q^{\alpha}} \left[\operatorname{erf}\left(\frac{q R_{\rm g}}{\sqrt{6}}\right) \right]^{3\alpha},\tag{S7}$$

with the Porod scaling factor C:

$$C = \frac{G\alpha}{R_{\rm g}^{\alpha}} \left[\frac{6\alpha^2}{(2+\alpha)(2+2\alpha)} \right]^{\alpha/2} \Gamma\left(\frac{\alpha}{2}\right).$$
(S8)

 $\Gamma(\frac{\alpha}{2})$ is the gamma function and G the Guinier scaling factor. Fig. S4 presents six examples of data fitted with the Beaucage model. The fit is performed in the q range [0.08, 1.03] nm⁻¹ by a non-linear least squares minimization. In particular, when regarding the bottom plot on the right hand side, the Beaucage model agrees very well with the experimental data, especially for the curvature of the signal in the low (i.e. ~[0.08, 0.2]) and high q regions (i.e. ~[0.3, 1]) which are very different, a behavior that is typical of polymer systems.

References

- Beaucage, G. Approximations Leading to a Unified Exponential/Power-Law Approach to Small-Angle Scattering. J. Appl. Crystallogr. 1995, 28, 717–728.
- Hammouda, B. Analysis of the Beaucage Model. J. Appl. Crystallogr. 2010, 43, 1474– 1478.



Figure S1: Integrated intensity data plotted against the magnitude of the scattering vector q and results of fit by Porod's law for six cells. The Porod exponent α and Porod constant K are also indicated for each cell and for the three regions of interest, heterochromatin/nucleoli (HC), euchromatin (EC) and cytoplasm.



Figure S2: a) $20 \times$ phase contrast micrograph of a cell, scale bar 50 μ m, with the corresponding scan area (black square). b) Dark-field contrast image of the cell shown in a. c) Map of the Porod exponent α and d) map of the Porod constant K of the cell shown in a using Porod' law as described in Eq. 2 of the main text. e) Histograms of the Porod exponent for the three ROIs and f) histograms of the Porod constant. g) Map of the surface-to-volume ratio $S_{\rm v}$. h) Gaussian fits of the Porod exponent distributions shown in e and g) Gaussian fits of the Porod constant distributions shown in f.



Figure S3: Gaussian fits of the Porod exponent α and Porod constant K distributions shown in Fig. 4 of the main text. The results of the fits (means and standard deviations) are presented in Tab. 1 of the main text.



Figure S4: Results of the Beaucage model fit for six cells for the three regions of interest, nucleoli/heterochromatin, euchromatin and cytoplasm.