Materials and methods

Models

We considered two models. In the first model (infinitely thin polygons), 16 freely jointed equilateral polygons having each 40 segments of length $l$ were confined within a sphere of radius $3.63l$. The confinement within the sphere was imposed by adding an energy

$$E_{\text{sphere}} = \begin{cases} 0 & \text{if all the vertex are inside the sphere} \\ \infty & \text{otherwise}. \end{cases}$$

We considered two cases: (i) Topologically unrestricted polygons with segments that were free to pass through each other and could result in the formation of knots and catenanes. (ii) Topologically restricted polygons, with segments that were not allowed to pass through each other. To model the binding to a transcription factory, eight polygons were attached together by setting an attractive interaction between eight preselected vertices, each one belonging to a different polygon (Figure S1). The attractive interaction between two vertices at position $r_i$ and $r_j$ was modelled by a harmonic potential

$$E_{\text{binding}} = C(r_i - r_j)^2.$$ 

By choosing $C = 100k_BT/l^2$, the average distance between the attached vertices was approximately $\approx 0.056l$ (Figure S2). The position of the transcription factory was defined as the centre of mass of the attached vertices. To control the topology of the configuration, segments of topologically restricted polygons where modelled as thin hard-core cylinders of radius $10^{-5}l$ (i.e. the distance between two non-consecutive segments had to be larger than $2 \times 10^{-5}l$). This was done by adding an energy

$$E_{\text{tubes}} = \begin{cases} 0 & \text{if distance between every pair of non-consecutive segments > } 2 \times 10^{-5}l \\ \infty & \text{otherwise}. \end{cases}$$

The model was therefore defined by the total energy

$$E = E_{\text{sphere}} + E_{\text{binding}}$$

for topologically unrestricted polygons and

$$E = E_{\text{sphere}} + E_{\text{binding}} + E_{\text{tubes}}.$$ 

for topologically restricted polygons.

In the second model (beaded chains), hard-core cylinders were replaced by beads of radius $l/2$ centred on the vertices of 16 freely jointed equilateral polygons having each 100 segments of length $l$. 

Each bead was modelled as a hard-core sphere, by adding an energy

\[ E_{\text{beads}} = \begin{cases} 0 & \text{if distance between every pair of non-consecutive vertices} \ > l \\
\infty & \text{otherwise.} \end{cases} \]

The beads were confined within the enclosing sphere of radius 12.6l by adding an energy

\[ E_{\text{sphere}} = \begin{cases} 0 & \text{if all the beads are inside the sphere} \\
\infty & \text{otherwise.} \end{cases} \]

As in the infinitely thin polygons model, the binding to a transcription factory was modelled by setting an attractive interaction between eight preselected vertices, each one belonging to a different polygon. The attractive interaction between two vertices at position \( r_i \) and \( r_j \) was modelled by a harmonic potential

\[ E_{\text{binding}} = C \left( |r_i - r_j| - l \right)^2. \]

By choosing \( C = 10k_B T/l^2 \), the average distance between the attached vertices was kept below 2l.

The model was therefore defined by the total energy

\[ E = E_{\text{sphere}} + E_{\text{binding}} + E_{\text{beads}}. \]

**Metropolis Monte Carlo algorithm**

In order to study the properties of the system in thermal equilibrium, we used the Metropolis Monte Carlo algorithm (1). This algorithm generates iteratively a sequence of configurations \( \{x_1, x_2, \ldots, x_N\} \) distributed according to the Boltzmann distribution.

Starting from a given configuration \( x_n \), a trial configuration \( x' \) is proposed. The trial configuration is accepted as the new configuration \( (x_{n+1} = x') \) with probability

\[ P(x_n, x') = \begin{cases} 1 & \text{if } E(x') < E(x_n) \\
\exp \left( \frac{1}{k_B T} (E(x_n) - E(x')) \right) & \text{otherwise.} \end{cases} \]

where \( E(x) \) is the energy of configuration \( x \). If \( x' \) is not accepted, \( x_n \) is chosen as the new configuration \( (x_{n+1} = x_n) \).

In our implementation of the algorithm, the trial configuration was generated from the configuration \( x_n \) by using a crankshaft move (2). A randomly chosen subchain was rotated around the line connecting the first and last vertex of the subchain by an angle randomly chosen with uniform distribution in the range \([\theta_{\min}, \theta_{\max}]\). For topologically unrestricted polygons \( \theta_{\min} = -\pi \) and \( \theta_{\max} = \pi \). For topologically restricted polygons \( \theta_{\min} \) and \( \theta_{\max} \) were chosen as the minimum and maximum angle such that the subchain did not cross any other segment (or bead) during the crankshaft rotation (Figure S3).
Initial configuration

To build the initial configuration (Figure S4), we started with 16 polygons in a regular 40-gone configuration stacked on top of each other. The Metropolis Monte Carlo algorithm with topologically restricted polygons described previously was then used with an energy

\[
E = E_{\text{tubes}} + E_{\text{central}},
\]

for the infinitely thin polygons model and

\[
E = E_{\text{beads}} + E_{\text{central}},
\]

for the beaded chains model. Here \(E_{\text{central}}\) was given by the sum of the squared distance from all the vertices to the centre of the sphere. The temperature was slowly decreased until all the polygons were inside the sphere. Note that this procedure generates configurations with all polygons unknotted and uncatenated.

Measured observables

Radial distribution of polygons with respect to the centre of the sphere:
The volume of the enclosing sphere (radius \(R_S\)) was divided in 40 equidistant shells with thickness \(R_S/40\) (Figure S5). The probability density for a given polygon was obtained by sampling 400 points equally spaced along the polygon (i.e. 10 point per segment), counting the number of these points within each shell and normalising the resulting distribution.

Radial distribution of attached vertices with respect to the centre of the sphere:
The probability density was computed in the same way as the radial distribution of polygons, except that there was only one vertex per polygon instead of 400 points.

Radial distribution of polygons with respect to their centre of mass:
Given a polygon, we wanted to characterize the distribution of its own points with respect to its centre of mass. To do this we started by evaluating its characteristic inertial ellipsoid (3). The volume enclosed within this ellipsoid was then divided in 40 concentric shells defined by the contours obtained by rescaling the characteristic inertial ellipsoid by factors \(2/40, 4/40, \cdots, 1, \cdots, 2\) (Figure S6). The probability density for a given polygon was obtained by sampling 400 points equally spaced along the polygon, counting the number of these points within each shell and normalising the resulting distribution. We used an ellipsoid instead of a sphere because it can better approximate the shape of a general polygonal configuration and is therefore more adequate to characterize whether a point is inside or outside a given chromosomal territory (Figure S7).

Radial distribution of attached vertices with respect to the centre of mass of the polygon to which they belong:
The probability density was computed in the same way as the radial distribution of polygons with respect to their centre of mass of the polygon, except that there was only one vertex per polygon instead of 400 points.

Contact probabilities:
Two vertices were considered in contact if the distance between them was smaller than a given distance \( d \). To evaluate the contact probability between two polygons, we considered all the pairs of vertices belonging to two different polygon. The contact probability between both polygons was evaluated as the ratio of the number of pairs which were in contact and the total number of pairs.
The contact probability between free polygons (denoted \( P_{\text{free,free}}(d) \)) was obtained by averaging the contact probabilities between all pairs of different free polygons. The contact probability between free and attached polygons (denoted \( P_{\text{free,attached}}(d) \)) was obtained by averaging the contact probabilities between all pairs of polygons with one free and one attached polygon.
For small \( d \), the contact probabilities \( P_{\text{free,free}}(d) \) and \( P_{\text{free,attached}}(d) \) are expected to scale as \( d^3 \) for the infinitely thin polygons model and \( (d - l)^3 \) for the beaded chains model, and as a consequence the ratio \( P_{\text{free,free}}(d)/P_{\text{free,attached}}(d) \) is expected not to depend on \( d \). This is indeed satisfied for \( 0.05l \leq d \leq 0.455l \), as shown in figure 5, which presents the ratio \( \langle P_{\text{free,free}}(d) \rangle / \langle P_{\text{free,attached}}(d) \rangle \) as a function \( d \). Here \( \langle \cdots \rangle \) denotes the thermal average.

Intermingling:
As in our previous work (4), we defined the intermingling between a set of polygons in the following way. We started by determining the intersection of individual polygons with the equatorial plane of the enclosing sphere. All the intersection points of a given polygon were then enclosed within the smallest convex envelope. The intermingling was then defined as the ratio between the area of intersection between convex envelopes \( A_I \) and the total area of convex envelope \( A_T \). More precisely, \( A_I \) is the area of the region defined as the set of points in the equatorial plane belonging to more that one convex envelope, while \( A_T \) is the area of the region defined as the set of points in the equatorial plane belonging to at least one convex envelope.
With this definition, intermingling values can range from 0 (no intermingling) to 1 (complete intermingling).
The intermingling between attached polygons was obtained by applying this procedure to the 8 polygons that were attached to each other. Similarly, the intermingling between free polygons was obtained by taking into account only the 8 polygons that were free.

Simulation run
For the infinitely thin polygons model, both topologically restricted and unrestricted systems were simulated during \( 2 \times 10^9 \) Monte Carlo iterations. After a first thermalization run of \( 10^6 \) iteration, the various observables were measured every 100 iterations, except for the radial distributions of attached vertices as well as the contact probabilities which were measured every
For the beaded chains model, the topologically unrestricted and restricted systems were simulated during $6.5 \times 10^9$ and $25 \times 10^9$ Monte Carlo iterations respectively. After a first thermalization run of $3.2 \times 10^6$ iteration, the various observables were measured every $16 \times 10^3$ iterations, except for the radial distributions of attached vertices as well as the contact probabilities which were measured every $1.6 \times 10^6$ iteration.

Let us denote by $\{A_1, A_2, \ldots, A_M\}$ the set of measurements for the observable $A$. For each observable $A$, we evaluated the average value

$$\langle A \rangle = \frac{1}{M} \sum_{i=1}^{M} A_i.$$  

The error bars for $\langle A \rangle$ were evaluated with the “blocking” method (also known as binning method) described by Flyvbjerg and Petersen (5). In order to have an idea about the number of iteration needed to decorrelate two configuration, we also evaluated the autocorrelation $C_A(k)$

$$C_A(k) = \frac{\langle A_{i+k} A_i \rangle - \langle A_{i+k} \rangle \langle A_i \rangle}{\langle A^2 \rangle - \langle A \rangle^2}$$

Convergence

A necessary condition for the Monte Carlo simulation to give meaningful results is that the number of iterations is large enough to obtain a statistically significant number of uncorrelated configurations. To check this condition, the number of iterations $n$ must be compared to a correlation time $\tau$, which can be defined as the number of iterations needed to decorrelate a configuration from the initial configuration. We don’t know how to measure $\tau$ directly, however it is possible to measure the correlation time $\tau_A$ for any observable $A$. A lowest bound for $\tau$ is then given by the maximum $\tau_A$. For the models studied here, the observables with the largest correlation time were the $x, y, z$ coordinates of the attached vertices. The autocorrelation of the $x$ coordinate (denoted by $C_{\text{Factory},x}(n)$) is shown in Figure S8 for both topologically restricted and unrestricted polygons (infinitely thin polygons model). A fit of $\exp\left(-n/\tau_{\text{Factory},x}\right)$ gives the correlation time $\tau_{\text{Factory},x} \approx 2.1 \times 10^7$ iterations for topologically unrestricted polygons and $\tau_{\text{Factory},x} \approx 2.6 \times 10^7$ iterations for topologically restricted polygons. Since the total number of iteration corresponds to approximately $95 \times \tau_{\text{Factory},x}$ for topologically unrestricted polygons and $75 \times \tau_{\text{Factory},x}$ for topologically restricted polygons, it suggest that the simulation generated enough uncorrelated configuration to obtain a correct evaluation of thermal equilibrium properties.

For the beaded chains model we obtained $\tau_{\text{Factory},x} \approx 4 \times 10^7$ and $\tau_{\text{Factory},x} \approx 6 \times 10^7$ for topologically unrestricted and restricted respectively, i.e. the total number of iteration corresponds to approximately $162 \times \tau_{\text{Factory},x}$ for topologically unrestricted polygons and $416 \times \tau_{\text{Factory},x}$ for topologically restricted polygons.
Another necessary condition for the Monte Carlo simulation to have converged is that the measured average values must satisfy the symmetries of the model. In particular the probability density of radial distance as well as the probability density of relative distance to the centre of mass must be the same for all equivalent polygons. This is indeed what we obtained when taking into account error bars.
References

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Topologically unrestricted | Topologically restricted
---|---
Attached polygons | 0.428 ±0.001 | 0.205 ±0.002
Free polygons | 0.330 ±0.002 | 0.102 ±0.002

**Table S1**: Intermingling between polygons that are attached together and between polygons that are free for the infinitely thin polygons model.

Topologically unrestricted | Topologically restricted
---|---
Attached polygons | 0.0231 ±0.0005 | 0.0163 ±0.0002
Free polygons | 0.0088 ±0.0003 | 0.0056 ±0.0002

**Table S2**: Intermingling between polygons that are attached together and between polygons that are free for the beaded chains model.

**Figure S1**: Eight polygons are attached together with attractive interactions (blue lines).
Figure S2: Distribution of the distance between attached vertices. The distance is in units of segment length $l$.

Figure S3: Crankshaft move in the case of topologically restricted polygons.
**Figure S4:** Initial configuration (a) used with the infinitely thin polygons model and (b) used with the beaded chains model. Polygons with striped texture are attached together.
Figure S5: Determination of the radial distribution of a polygon with respect to the centre of the sphere. For clarity only 10 spherical shells are shown. This figure is only a cartoon intended to explain the method and does not present the actual data. The actual data are presented in Fig. 1 c and d.
Figure S6: Determination of the radial distribution of a polygon with respect to its centre of mass. For clarity only 10 shells inside the characteristic inertial ellipsoid (in blue) are shown. This figure is only a cartoon intended to explain the method and does not present the actual data. The actual data are presented in Fig. 2 d and e.
**Figure S7:** An ellipsoid can better approximate the general shape of a chromosomal territory than a sphere. Consider a point (black circle) on the periphery of a territory (grey region). In a spherical approximation (a), the point will be deep inside the approximated territory (thick blue line), while with an ellipsoidal approximation (b) it will appear close to the periphery of the approximated territory (thick blue line).

**Figure S8:** Autocorrelation of the factory position $x$ coordinate $C_{\text{Factory},x}(n)$ for topologically unrestricted (red circles) and restricted (blue circles) polygons. The red and blue lines are fit of $\exp\left(-n/\tau_{\text{Factory},x}\right)$ to $C_{\text{Factory},x}(n)$.