Segregation of polymers in confined spaces

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Abstract
We investigate the motion of two overlapping polymers confined in a 2D box. A statistical model is constructed using blob-free-energy arguments. We find spontaneous segregation under the condition \(L > R_{∥}\), and mixing under \(L < R_{∥}\), where \(L\) is the length of the box and \(R_{∥}\) is the polymer extension in an infinite slit. The segregation time \(\tau\) is determined by solving a mean first-passage time problem and by performing Monte Carlo simulations. Both show a minimum in \(\tau\) as a function of \(L\). Although our results are restricted to 2D, the basic mechanism of competition between entropy and confinement leading to the minimum is suggestive of an evolutionary driving force for size selection.

Biopolymers have evolved to function in crowded and confined environments [1]. Under these conditions, excluded volume effects and geometrical confinement compete with entropy to yield unique structures and dynamical processes [2–5]. Examples include the transportation of proteins through a membrane channel and endoplasmic reticulum, and the replication and segregation of highly compacted chromosomes during cell division [6–8].

The mechanism underlying the process of chromosome segregation in bacterial cells is of central importance to cell biology and still unclear [3, 9–12]. Recent results of molecular dynamic simulations of flexible polymers indicate that entropic driving forces, in the absence of motor proteins, can describe some important features of chromosome segregation in \(C.\) crescentus and \(E.\) coli [3, 13]. Analytical calculations of the dynamics in an open cylinder predict a constant segregation velocity [14]. Experimental studies of the sequential movement of chromosomal loci during replication, however, show that the segregation process is quick and inhomogeneous [13, 15, 16]. Analysis of segregation in a closed geometry [17] indicates that the shape of the confining box determines whether polymers segregate or remain mixed. A natural question that arises is whether the inhomogeneous dynamics observed in experiments is a consequence of the shape of the cell.

In this letter, we analyze the motion of two identical, self-avoiding chains each with \(N\) segments of Kuhn length, \(a\), and confined to a 2D box of width \(W\) and length \(L\) (figure 1(a)). The width is chosen to be less than \(R_g\), the radius of gyration of the free polymer and larger than Kuhn length. Combining scaling theory, stochastic models and Monte Carlo simulations, we show the existence of a transition from segregation to mixing with the change of the aspect ratio. The dynamics of segregation is shown to be inhomogeneous, with two distinct regimes. The segregation time sensitively depends on the geometry, and interestingly, exhibits a minimum at a specific aspect ratio that depends on the density. Our model in 2D provides a consistent picture of the segregation dynamics in the absence of complicated 3D effects such as entanglement and knots, which differentiates the influence of entropy and confinement on the segregation timescale from intrinsically 3D effects and distinguishes which factors enter what aspect of the dynamics.

Blob scaling
A well-known scaling theory of the free energy of confined polymers is based on the concept of a self-consistent structure, a blob of monomers, each of which contributes \(k_BT\) to the free energy of confined polymers (figure 1(a)) [18–20]. The blob size, \(\xi_{blob}\), is determined by the competition between confinement, excluded volume effects and entropy. For length scales smaller than \(\xi_{blob}\), confinement effects are screened and self-avoidance effects dominate, but for length scales larger...
than \( \xi_{\text{blob}} \), polymer conformations are strongly affected by the confining geometry. Two basic assumptions enter the blob-scaling picture: a homogeneous distribution of monomers, and Flory scaling of \( \xi_{\text{blob}} \) with \( g \), the number of segments in a blob [18]. For a single chain confined in the same rectangular geometry (width \( W \) and length \( L \)) as shown in figure 1(a), the blob size is given by [18–20]

\[
\xi_{\text{blob}} \propto \left( \frac{WL}{N^\nu} \right)^\frac{1}{\nu},
\]

where \( \nu = \frac{3}{4} \) is the Flory exponent in 2d [18, 19]. In the following, all lengths will be measured in terms of \( a \). The extension of the polymer in the longitudinal direction, \( R_\parallel (\xi_{\text{blob}}) \), obtained by assuming that the blobs form a chain scales as \( 1/\xi_{\text{blob}} \) [18, 19]. Consequently, the monomers are homogenous with the density \( \frac{N}{WR_\parallel (\xi_{\text{blob}})} \). The width of the box provides an upper bound for \( \xi_{\text{blob}} \), defining a length scale \( R_\parallel = R_\parallel (W) \propto NW^{-\frac{1}{2}} \) [18]. For \( L > R_\parallel \), \( \xi_{\text{blob}} = W \), blobs occupy only a small portion of the confining space, and the situation is similar to an open slit. For \( L < R_\parallel \), \( \xi_{\text{blob}} < W \), and \( R_\parallel (\xi_{\text{blob}}) = L \). In this regime, the 2D nature of the confinement significantly affects blob statistics, making it Gaussian. We introduce a dimensionless parameter \( \lambda = \frac{2R_\parallel - L}{R_\parallel} \) to identify different confinement regimes: \( \lambda \geq 1 \), 2d confinement, \( 0 \leq \lambda \leq 1 \), open slit and \( \lambda < 0 \) (\( L \geq 2R_\parallel (W) \)), open-slit for two confined polymers.

Since each blob contributes \( \sim k_B T \equiv 1/\beta \) to the free energy of a single polymer [18, 19], \( F(L, W, N) \):

\[
\beta F(L, W, N) \propto N_{\text{blob}} = \begin{cases} 
\frac{N^3}{(LW)^\frac{3}{2}} & \lambda \geq 1 \\
\frac{N^3}{(R_\parallel W)^\frac{3}{2}} = NW^{-\frac{1}{2}} & 0 \leq \lambda < 1,
\end{cases}
\]

where \( N_{\text{blob}} = N/g \) is the number of blobs in a single confined polymer.

**Segregation**

In order to model the segregation of two polymers in a confined geometry, we extend the blob scaling argument by retaining the homogeneity assumption so that the monomers are uniformly distributed in the occupied space. We also impose that the extension of two overlapping polymers confined in a narrow slit \( (R_\parallel) \) is the same as that of a single polymer \( (R_\parallel)^1 \) since the thickness of chains is infinitesimal, as illustrated in figure 1(a).

In the later simulations, we find that the difference of average extension between single chain and two overlapping chains is less than 6% [21], supporting the assumption that the extension remains unchanged. We note that the assumption is consistent with recent studies that the difference of extension between a single chain (N-monomer) and ring polymer (2N-monomer) confined to a nanochannel is about 10% since the ring polymer is very similar to our case of two overlapped polymers [22]. Using homogeneity, the linear density of segments for two overlapping polymers is \( \phi = \frac{2N}{4(R_\parallel)^2} \) for \( \lambda \geq 1 \) (\( \lambda \leq 1 \)). During the process of segregation, the box space is naturally partitioned into three regions: region I of length \( L \), \( 0 \leq l \leq R_\parallel \) occupied by two polymers, and the symmetry-equivalent regions II and III, occupied by single-polymer segments (figure 1(b)). The total free energy of the polymers is \( F(l) = F_o + 2F_l \), where \( F_o \) (\( F_l \)) denotes the free energy in the overlapping (single-chain) regions. If the dominant mechanism of segregation is a lateral sliding of the polymers with negligible transverse displacements, then the linear density in region I remains fixed at \( \phi \), defining the monomer densities \( \phi_1 = \phi, \phi_2 = \phi_3 = \frac{2N - \phi l}{4(l - l_0)} \).

Blob sizes can be calculated by combining these linear densities and equation (1). For \( \lambda \geq 1 \), the blob size, \( \xi_{\text{blob}} = (Wl)^{\frac{1}{2}} \), does not differ from region to region. Using equation (2), we can obtain

\[
F_o = F(l, W, \phi l) \quad F_l = F \left( \frac{L - l}{2}, W, \frac{2N - \phi l}{2} \right).
\]

The total free energy is obtained using the expression of linear densities and equation (3). For \( \lambda \geq 1 \), \( \beta F(l) \propto \frac{3N^3}{2W^2} \) is independent of \( l \), and therefore, there is no force driving segregation [20]. For \( 0 \leq \lambda \leq 1 \), \( \xi_{\text{blob}} \) is \( 2^{-1.5}W \) and \( 2^{-1.5}W(\frac{2R_\parallel}{L})^{1.5} \) in region I and region II (III), respectively, and

\[
\beta F(l) \propto \begin{cases} 
\frac{2}{W}(R_\parallel + 3l) & \text{if } 2R_\parallel - l \leq l \leq R_\parallel \\
\frac{8}{W} \left[ \frac{(R_\parallel - l)^3}{(L - l)^2} + l \right] & \text{if } 0 \leq l \leq 2R_\parallel - L.
\end{cases}
\]

Since \( L \geq R_\parallel \), blobs in region I are smaller than those in regions II (III) (figure 1(b)). For \( \lambda \leq 0 \), \( \beta F(l) \propto \frac{d}{dl} \) is identical to the free energy of two chains in an open tube [14], and the longitudinal confinement has no effect.

For all \( \lambda \leq 1 \), \( F(l) \) is a monotonically increasing function of \( l \), and this repulsive potential drives segregation. Assuming \( F(l) \) is continuous with respect to \( l \), and also continuous for the entire range of \( \lambda \).
that the segregation time is long compared to the polymer relaxation time, the long-time dynamical behavior of the segregating polymers \((L \geq R_i)\) can be modeled as equilibrium dynamics controlled by \(F(I)\). The global relaxation time of the end-to-end distance of a confined polymer grows less rapidly than \(N^2\) \[23, 24\]. Since the segregation time for \(\lambda \approx 1\), grows faster than \(N^2\), the equilibrium assumption should hold \[25–27\]. Independent simulations have been performed to directly measure the equilibration time, and results support the assumption \[21\].

**Dynamics**

The segregation time \(\tau\) is defined as the average time for chains moving from \(l = R_i\) to \(l = 0\), which is equivalent to the mean first-passage time of the Fokker–Planck equation \[25, 26, 28\]:

\[
\frac{\partial P(l,t)}{\partial t} = \frac{\partial}{\partial l} D e^{\beta F(l)} \frac{\partial}{\partial l} e^{\beta F(l)} P(l,t),
\]

where \(P(l,t)\) is the probability of the two chains overlapping by \(l\) at time \(t\), \(D\) is the diffusion constant, \(D = \frac{k_B T}{\xi}\) and \(\xi\) is the monomer friction coefficient \[26, 27\]. Since \(F(l)\) is a piecewise continuous function with different expressions in regions I and II (III), the mean first-passage time can be written as the sum of the first-passage times of two subprocesses: \(\tau = \tau_1 + \tau_2\), where \(\tau_1\) is for the subprocess of separation between \(l = R_i\) and \(l = 2R_i - L\) due to the entropy, and \(\tau_2\) is for separation between \(l = 2R_i - L\) and \(l = 0\) due to the competition between the entropic and longitudinal pressure \[29, 30\].

Introducing \(x \equiv R_i - l\), we obtain modulo constant factors

\[
\tau_1 = \frac{1}{D} \int_0^{L-R_i} e^{\beta F_1(x)} \int_0^x e^{\beta F_2(y)} dy dx
\]

\[
\tau_2 = \frac{1}{D} \int_{L-R_i}^{R_i} e^{\beta F_1(x)} \int_{L-R_i}^x e^{\beta F_2(y)} dy dx,
\]

where \(\beta F_1(x) = -\frac{6}{w} x\) and \(\beta F_2(x) = \frac{8}{w} \left(\frac{x^3}{(L-R_i+x)^3} - x\right)\) \[26, 30\].

In the limit of \(\lambda \approx 0\) (\(L \approx 2R_i\)), the segregation time is controlled by \(\tau_1\):

\[
\tau \approx \tau_1 = \frac{W^2}{36D} \left[e^{-\frac{6(L-R_i)}{W}} + \frac{6(L-R_i)}{W} - 1\right]
\]

\[
\approx \beta \xi WN(L-R_i).
\]

Simulations

The theoretical predictions for segregation dynamics are based on scaling arguments and an assumption of quasi-equilibrium dynamics. Although the individual assumptions entering the theoretical framework are difficult to test, the predictions can be tested in simulations and experiments.

We performed Monte Carlo simulations based on the bond fluctuation model (BFM) \[31, 32\] for the segregating model analyzed theoretically. The BFM is a coarse-grained model in which polymer chains live on a hypercubic lattice, fluctuations on scales smaller than the lattice constant are suppressed and the effective monomers are connected by bonds constructed to account for excluded-volume effects. An overlapping configuration of two chains is created by introducing a pseudo-harmonic interaction: \(\sum_{i=1} k(R_{ij} - R_{ij})^2\), where \(R_{inn}\) denotes the position vector of the \(n\)th monomer on the \(n\)th polymer and \(k\) is a parameter controlling the attractive strength. This interaction is turned off after the two chains are fully relaxed in the overlapped configuration. The separation between two chains is measured by the horizontal \((X_{cc})\) and vertical \((Y_{cc})\) projections of the centers of mass, and \(X_{cc}\) is related to \(l\):

\[
X_{cc} = \begin{cases} 
\frac{1}{2}(L-l) & \text{if } 0 \leq l < 2R_i - L \\
R_i - l & \text{if } 2R_i - L < l < R_i.
\end{cases}
\]

Simulations were performed over a wide range of parameters: \(80 < N < 200, 30 < L < 140\), keeping \(W = 10\) fixed. Each Monte Carlo trajectory spans a few hundred Rouse times \[18, 31\], and 50 independent trajectories are sampled for each set of parameters to obtain accurate statistics.

Figure 2 illustrates the evolution of \(X_{cc}\) and \(Y_{cc}\) with respect to Monte Carlo steps (MCS). For \(\lambda = 0.39, 0.65, 0.66, X_{cc}\) grows and fluctuates around \(L/2\) and \(Y_{cc}\) decreases to zero, a signature of segregation (figure 2(a)). The timescale for reaching a well-defined average is the same for \(X_{cc}\) and \(Y_{cc}\), which is a convincing argument for equating this measured time to the calculated segregation time, \(\tau\). In the simulation, \(\tau\) is defined as the first time \(X_{cc}\) reaches \(\frac{L}{2}\) for the segregation case. Figure 2(b) shows that for \(\frac{L}{2} > 1.08\), \(X_{cc}\) and \(Y_{cc}\) do not grow but fluctuate between 0 and \(\frac{L}{2}\) (\(X_{cc}\)), and between 0 and \(\frac{L}{2}\) (\(Y_{cc}\)) even after \(10^3\) MCS \[21\], indicating a lack of segregation. In figure 3(a), Monte Carlo simulation results and numerical integrals of equation (6) are shown; both demonstrate a minimum in \(\tau\) as a function of \(L\). The minimum is much better defined in the simulations. Figures 3(b) and (c) illustrate the validity of the asymptotic scaling of \(\tau\) predicted by equations (7) and (8). The corresponding parameters used for data points from left to right in figures 3(b) and (c) are listed in tables 1 and 2, respectively.

Figure 4 shows simulation data and the theoretical predicted phase boundary separating the segregated and the mixed phase. The geometry with minimal segregation time is marked (figure 4).

Ring polymers such as the chromosome of *E. coli* are distinct from their linear counterpart since they form a close loop. Topologically, they have double-stranded configuration while confined in the small cylinder. Therefore, a minimal
Table 1. Parameters for large $\lambda$.

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| $N$ | 80 | 100 | 120 | 150 | 180 | 200 | 200 | 200 |
| $L$ | 35 | 45 | 55 | 65 | 70 | 80 | 85 | 85 | 90 |
| $\lambda$ | 0.83 | 0.85 | 0.8 | 0.92 | 0.78 | 0.89 | 0.79 | 0.95 | 0.84 |

Table 2. Parameters for small $\lambda$.

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| $N$ | 80 | 100 | 100 | 120 | 150 | 180 | 180 | 200 | 200 | 200 | 200 |
| $L$ | 60 | 70 | 80 | 90 | 110 | 130 | 140 | 140 | 150 | 160 |
| $\lambda$ | 0.068 | 0.19 | 0.04 | 0.1 | 0.13 | 0.16 | 0.08 | 0.18 | 0.11 | 0.036 |

Figure 2. (a) Plot of $2X_{cc}/L$ versus MCS in the segregation region for ($\lambda = 0.39; N = 100, L = 60$), ($\lambda = 0.65; N = 120, L = 65$) and ($\lambda = 0.66; N = 200, L = 100$). The inset shows $Y_{cc}$ versus MCS for $\lambda = 0.39$. (b) Plot of $2X_{cc}/L$ and $2Y_{cc}/L$ versus MCS in the mixing region: ($\lambda = 1.08; N = 100, L = 40$).

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extension of the blob picture to 3D ring polymers confined into a closed cylinder is to require $\xi_{blob} \leq W^2$ rather than $W$ [21]. For ring polymers, we expect an additional repulsion due to topological constraints [3, 33, 34]; however, we do not expect this to be strongly dependent on the geometrical confinement. Therefore, this extra repulsion changes the qualitative behavior of the segregation time. Combining the assumptions that the segments perform a self-avoiding random walk inside of each blob and that the blobs are closely packed in the cylinder [19], one can obtain the longitudinal extension $R_\parallel^L \approx NW^{-2}\xi_{blob}^{3-\frac{1}{2}}$. Applying this model to E. coli, using measured parameters [17, 3, 35]: $\xi_{blob} \approx 87$ nm, $W \approx 0.24$ nm and $L \approx 1.39 \mu$m, locates E. coli in the segregation phase and close to the geometrical condition of minimum segregation time. While this observation may be fortuitous since a chromosome strand is immensely more complicated than a linear polymer, it raises the interesting possibility that genome segregation times could have applied evolutionary selection pressure to genome lengths.

In conclusion, a theoretical framework, based on the blob picture to capture the essence of the competition between excluded volume and confining effects, predicts a rich phenomenology of transitions between segregated and mixed states and optimal geometries that minimize the segregation time. Monte Carlo simulations provide broad support for the theoretical predictions. Experiments in microfluidic devices should be able to provide direct tests of the predictions and elucidate the role of entropy in driving segregation of biopolymers. The simulation can be extended to study more realistic models based on actual chromosomes structure of...
Figure 4. Phase diagram of segregation and mixing. The x-axis parameterizes the monomer concentration and the y-axis parameterizes the geometry. Simulation data are denoted by filled symbols (segregation) and open triangles (mixing). Filled symbols illustrate data from various $L/N$ values: 80 (circle), 100 (triangle), 120 (inverted triangle), 150 (hexagon), 180 (tetragon) and 200 (pentagon). Stars mark aspect ratios with the minimal segregation times. The purple square denotes an estimate based on experimental data for *E. coli*.

bacteria. In addition, the details of segregation including the scaling relation of segregation time can be explored more accurately by using finite-size scaling in the probability distribution of $X_{cc}$ [36].

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