Timescale of entropic segregation of flexible polymers in confinement: implications for chromosome segregation in filamentous bacteria

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We report molecular dynamics simulations of the segregation of two overlapping chains in cylindrical confinement. We find that the entropic repulsion between the chains can be sufficiently strong to cause segregation on a time scale that is short compared to the one for diffusion. This result implies that entropic driving forces are sufficiently strong to cause rapid bacterial chromosome segregation.

I. INTRODUCTION

Confined polymers play an important role in many industrial processes and biological systems. Examples range from membrane filtration and oil recovery to gel electrophoresis and protein translocation [1,2,3]. Importantly, recent technological development in nano-/microfluidics has made it possible to manipulate and trap biomolecules such as double-stranded (ds) DNA in confined environments with a characteristic lengthscale that is much smaller than the radius of gyration of the polymers [4,3,6]. Also under biological conditions, DNA is often strongly confined, e.g., packed into a viral capsid [7], bacteria [8] or the eukaryotic cell nucleus [9].

In this article, we report molecular dynamics simulations that allow us to determine the typical speed of the segregation of initially mixed polymers in cylindrical confinement. This problem has particular relevance for the understanding of chromosome segregation in bacteria, where the nature of its underlying mechanism is currently under debate. Here, the basic issue is whether the major driving force for segregation of duplicating chromosomes in strong confinement is physical (driven by entropic or mechanical “pushing”) [8,10] or biological (such as cytoskeletal and motor proteins) [11,12].

Our results show that the effective repulsion between two chains in a cylindrical geometry of confinement can be very strong. Typically, the segregation requires a time proportional to $N^2$, which is much faster than the $N^3$ timescale of chain diffusion, where $N$ is the chain length. This suggests that for filamentous bacteria such as Strep-tomyces coelicolor [13] or cyanobacterium Anabaena [14], the main driving force of chromosome segregation might be entropic and any additional mechanisms are for “optimization.” As we shall discuss later, our proposal is fully consistent with the recent results that chromosome segregation in some filamentous bacteria is a random pro-

\[ F_{\text{eff}} = -\frac{\partial F}{\partial R_{c2c}} = \frac{k_B T}{D}, \]

and, thus, the equation of motion for the center of mass is

\[ M \ddot{R}_{c2c} = \frac{k_B T}{D} - \Gamma V_{c2c}. \]
where $M = Nm$ and $\Gamma$ are the total mass and the effective friction of the chain, respectively ($m$ is the mass of a single monomer). Ignoring hydrodynamic interactions between monomers, one can assume that the frictions $\gamma$ on the individual monomers are additive, i.e., also $\Gamma = N\gamma$. Then, the solution of Eq. [2] with an initial condition $V_{c2c} = 0$ at $t = 0$ can be obtained as

$$V_{c2c}(t) = \frac{k_BT}{\gamma DN}(1 - e^{-\frac{t}{\tau^*}}),$$  \hspace{1cm} (3)

where $\tau^* = m/\gamma$ is the “inertial” timescale. In practice, $t \gg \tau^*$ and hence the characteristic segregation speed is constant and given by

$$V_{c2c} \sim \frac{k_BT}{\gamma DN}.$$  \hspace{1cm} (4)

The equilibrium length of confined chains, $L_{eq}$, is proportional to $N$. Therefore, the time for reaching complete segregation, $R_{c2c} = L_{eq}$, scales as

$$t_s \sim L_{eq}/V_{c2c} \sim N^2.$$  \hspace{1cm} (5)

This is time is much shorter than $t_{diff}$, the typical time it takes a single chain to diffuse over a distance equal to its own length:

$$t_{diff} \sim \frac{L^2_{eq}}{2D_{diff}} \sim N^3.$$  \hspace{1cm} (6)

However, the above considerations do apply for the initial situation of complete overlap, $R_{c2c} \approx 0$. In this case the system is in a state of unstable equilibrium, since the effective segregation force is $F_{eff} \approx 0$. Hence, the system will initially show purely diffusive behaviour until a certain separation, typically $R_{c2c} \approx D$, is reached. We refer to the time until segregation sets in as the “induction time” $t_i$ that should scale as $N^3$. With increasing $D$, diffusion becomes easier, because the monomer concentration decreases, and $t_i$ decreases, while $t_s$ increases with $D$. Below, we show that for all practically relevant diameters, the segregation process is rate limiting.

For real bacteria, entropic segregation already sets in during replication. Therefore, segregation always takes place for comparatively short pieces of DNA, so that the induction time does not play a role.

### III. SIMULATION METHOD

In the molecular dynamics simulations, we model the polymers using a bead–spring model in a cylindrical compartment of diameter $D$; each chain consists of $N$ beads of diameter $\sigma$. The bead–bead and bead–compartment interactions were modeled by a Weeks-Chandler-Andersen potential (WCA) [17], which corresponds to the repulsive part of the Lennard-Jones potential:

$$U_{\text{WCA}}(r) = \epsilon_{\text{WCA}} \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^{6} + \frac{1}{4} \quad \epsilon_{\text{WCA}}$$  \hspace{1cm} (7)

TABLE I: The simulation parameters for the different runs.

| $N$ | $D$ | $L_{eq}$ | $L_{eq}$ |
|-----|-----|---------|---------|
| 100 | 1.5 | 72.5 | 64.4 |
| 200 | 1.5 | 146.0 | 130.3 |
| 300 | 1.5 | 219.5 | 188.3 |

The first three tables give for different chain lengths $N$ the simulated pore diameters $D$ and the corresponding equilibrium end-to-end distances $L_{eq}$ of a single chain. The last table contains the number $T_{\text{warm}}$ of timesteps used for equilibration of the interconnected chains, the number of timesteps $T_{\text{config}}$ between recorded configurations, and the number $N_{\text{config}}$ of independent simulation runs with different random seeds.

for $r < \sqrt{2}\sigma$ and 0 elsewhere. $r$ denotes the distance between two bead centers for the bead–bead interactions, and the distance between the bead center and the compartment minus $\sigma$ for the bead–compartment interactions. At $r = \sigma$, the interaction energy is $\epsilon_{\text{WCA}} = k_BT$; since the potential is quite steep, $r$ will typically stay above 0.9$\sigma$. This models soft beads of diameter $\sigma$, whose centers cannot come much closer than $\sigma$ to each other, and cannot penetrate the wall (i.e. the wall imposes a constraint on the sphere centers, as depicted in Fig. 1). In the simulation, $\sigma$ defines the basic length scale and $\epsilon_{\text{WCA}}$ the energy scale. Our unit of mass is given by $m$, the mass of a bead. We choose the temperature such that $k_BT/\epsilon = 1$. Having specified our basic units, the time unit is given by $\tau_{\text{WCA}} = \sigma/m/\epsilon_{\text{WCA}} = 1$. In the following, we will omit these units.

The springs between the beads in a chain were formed by the FENE (finite extensible nonlinear elastic) potential

$$U_F(r) = -\frac{1}{2} \epsilon_F r_F^2 \ln \left( 1 - \left( \frac{r}{r_F} \right)^2 \right),$$  \hspace{1cm} (8)

where $r$ is the distance of the bead centers, $r_F$ is the radius at which the potential becomes singular, and $\epsilon_F$ is the interaction strength. In the present simulations, we chose $\epsilon_F = 10$ and $r_F = 2$. In combination with the WCA potential this results in a typical bond length of 1.027.

We simulate this system using the simulation package ESPResSo [18]. To propagate the system, we employ a velocity-Verlet MD integrator with a fixed time step of 0.01; the system is kept at constant temperature by means of a Langevin thermostat with a fixed friction of $\gamma = m\tau_{\text{WCA}}$, so that $\tau^* = \tau_{\text{WCA}} = 1$. Other parameters
FIG. 2: Example simulation run for $D = 7$, $N = 200$, starting from the removal of the interconnecting bonds. The two gray bands give the total extents along the tube axis of the two chains for a single run, the black lines the positions of their centers of mass. The positions are relative to the total center of mass $R$ of the system and rescaled by the equilibrium length $L_{eq}$ of a single confined chain.

We continue to simulate the system until the two chains have segregated, i.e., until the chains do not overlap and their centers of mass are separated by at least the equilibrium length $L_{eq}$ of a single chain, which had been determined beforehand by separate simulations. During this run, we record configurations every $T_{config}$ simulation steps.

FIG. 3: Schematic view of the segregation process. A line is fitted to the center of mass distance $R_{c2c}$ in the range from $D$ to $L_{eq} - D$. From this fit, the induction time $t_i$ is determined as intersection with $R_{c2c} = 0$, and the segregation speed $V_{c2c}$ as its slope. The segregation time is then $t_s = L_{eq}/V_{c2c}$.

This procedure is repeated $N_{config}$ times (see Table I), resulting in $N_{config}$ independent data sets similar to Fig. 2. For each of these data sets, we calculate the distance $R_{c2c}(t)$ of the centers of mass of the two chains parallel to the cylindrical compartment as a function of time. Initially, $R_{c2c}$ is zero due to the preparation of the system, and stays close to zero during the induction time. Eventually, segregation sets in, and $R_{c2c}$ grows rapidly until $R_{c2c} = L_{eq}$ is reached, at which time the chains do not overlap anymore. Further increase in $R_{c2c}$ is only due to diffusion and is therefore much slower.

Fig. 3 displays schematically how we extract the induction and segregation times from each run: we fit a linear function $(t - t_i)V_{c2c}$ to the range in which $R_{c2c}(t)$ is between $D$ and $L_{eq} - D$. Here, $t_i$ is the extrapolated onset time of segregation and $V_{c2c}$ is the speed with which the two centers of mass separate in the linear regime. We always find linear segregation behavior for $D \leq R_{c2c} \leq L_{eq} - D$. The lower limit implies that the chains are separated by at least one blob diameter, the upper limit guarantees that there is at least one blob-size overlap left.

**IV. RESULTS AND DISCUSSION**

As can be seen in Fig. 4, our simulation clearly support the scaling prediction $V_{c2c} \sim 1/(ND)$ (Eqn. (3)). The prediction that the segregation time, scales as $N^2$ is only recovered for small tube diameters. This is not unexpected, because when $D$ approaches $L_{eq}$, the simple blob prediction for $L_{eq}$ breaks down [19] and the segregation time levels off at the relaxation of a free chain. The segregation speed relation Eqn. (4) however seems to be quite robust even for finite systems.

The measured average induction time $t_i$ is shown in...
Fig. 4: Measured segregation speed $V_{c2c}$ rescaled by $1/N$. For better visualization, we actually plot its inverse, $1/(NV_{c2c})$. The dashed line demonstrates the linear scaling of the segregation time with $D$. The inset shows the segregation time $t_s$ rescaled by $N^2$, as a function of $D$.

Fig. 5: Measured induction times $t_i$ rescaled by $N^3$. The lines are just guides to the eye.

Fig. 6: Monomer density $\rho(r)$ along the cylinder-axis for $N = 200$, $D = 8$, averaged over all configurations with a center of mass distance $R_{c2c} = 0$ (a), $R_{c2c} = 20$ (b), $R_{c2c} = 48$ (c) and $R_{c2c} = 80$ (d). The graphs are centered around the systems center of mass $R$.

V. CONCLUSIONS

Our simulations support the scaling prediction that the entropically driven segregation of two confined chains requires a time proportional to $N^3$. For long chains, this time is much shorter than the diffusive time that scales as $N^3$. We stress that this speed up of entropically driven segregation does not involve any active (energy-consuming) process. Considering the geometry of confinement and the length scales of (filamentous) bacteria, our results strongly suggest that the partitioning of duplicated chromosomes in these organisms is, at least partly, entropy-driven. Since the segregation sets already in during replication, there is no initial “induction” regime for bacteria. Indeed, the recent data obtained by Hu et al.
on the cyanobacterium *Anabaena* sp. PCC 7120 suggests that MreB, a bacterial actin homologue that is speculated by some as a “track” for transporting chromosome by putative motor proteins, is important for cell shape but not for chromosome segregation \[14\]. Moreover, they also have shown that the ratios of DNA content in two daughter cells have a much wider distribution than in the case that the two cells were identical. This suggests that chromosome partitioning is a random process, in good agreement with our entropy-driven segregation process we presented in this article.

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