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A polymer in a crowded and confined space: effects of crowder size and poly-disperity†

Juin Kim, Chanil Jeon, Hawoong Jeong, Youngkyun Jung, and Bae-Yeun Ha

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DNA compaction in a bacterial cell is in part carried out by entropic (depletion) forces induced by “free” proteins or crowding particles in the cytoplasm. Indeed, recent in vitro experiments highlight these effects by showing that they alone can condense the E. coli chromosome to its in vivo size. Using molecular dynamics simulations and a theoretical approach, we study how a flexible chain molecule can be compacted by crowding particles with variable sizes in a (cell-like) cylindrical space. Our results show that with smaller crowding agents the compaction occurs at a lower volume fraction but at a larger concentration such that doubling their size is equivalent to increasing their concentration fourfold. Similarly, the effect of polydispersity can be correctly mimicked by adjusting the size of crowders in a homogeneous system. Under different conditions, however, crowding particles can induce chain adsorption onto the cylinder wall, stretching the chain, which would otherwise remain condensed.

1 Introduction

The bacterial chromosome, macroscopically long along its backbone (∼2 mm for E. coli), occupies an intracellular space of micron size only, known as the nucleoid. How can this be accomplished in concert with other cellular or subcellular processes in the cell such as chromosome segregation and cell division, possibly without introducing much redundancy? Cells are crowded with biomolecules such as proteins and RNA (for an ‘artist impression’ of crowdedness, see Refs. 3,4). For instance, a typical E. coli cell contains ∼10^6 cytoplasmic proteins, occupying a large (∼20%) fraction of the cell volume. While the complete picture of chromosome compaction is still elusive, it has often been attributed to three distinct factors: nucleoid-associated proteins, DNA supercoiling, and entropic (depletion) forces induced by “free” proteins and other crowding agents available in the cell (see Refs. 1,2,8–12 for depletion forces in more general contexts).

On the other hand, recent single molecule experiments suggest that depletion forces alone can condense the E. coli chromosomes to its in vivo size, highlighting the favorable effects of molecular crowding once labelled as ‘obvious but under-appreciated.’

A few biological uncertainties make it challenging to interpret the compaction experiments. First, the E. coli chromosome is structurally heterogeneous. This might be responsible for the observed coexistence between condensed and extended phases, which would otherwise be interpreted as a signature of abrupt transitions. Indeed, chain collapse appears to be initiated preferentially at some region in the chromosome during the repeated compaction-decompaction cycle (see Movie S5 in Ref. 16). Consideration of a simpler system, i.e., a polymer in a confined and crowded space, will be beneficial for clarifying the role of molecular crowding in condensing a chain molecule and thus possibly for advancing our understanding of chromosome compaction in a cell.

Here, we carry out molecular dynamics simulations of a self-avoiding polymer in a crowded and confined space as a coarse-grained model of the bacterial chromosome that captures only the essential features of chain molecules in a crowded space: chain connectivity as well as the excluded volume of chain segments and crowders. We treat monomers and crowders on an equal footing (both explicitly). Here, a large degree of coarse-graining is inevitable for various reasons. First, the physical properties of chromosomes have only begun to be revealed (see Refs. 16–18 for recent progress). Also, the explicit treatment of crowders interacting with a long polymer is computationally demanding. Indeed, the degree/nature of coarse-graining in a physical approach to a biological system should reflect the desired level of abstraction vs. specificity.

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a Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea. Email: hjjeong@kaist.edu
b Department of Physics and Astronomy, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1. E-mail: byha@uwaterloo.ca
c Institute for the BioCentury, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea.
d APCTP, Pohang, Gyeongbuk 790-784, Korea. Email: hjjeong@kaist.edu
e National Institute of Supercomputing and Networking, Korea Institute of Science and Technology Information, Daejeon 305-806, Korea. E-mail: yjung@kisti.re.kr

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As evidenced later, this much simplified model leads to a few experimentally-testable predictions, which we believe will be useful for clarifying the role of depletion forces in organizing chromosomes in a confined cell-like space.

In our work, each monomer represents a structural unit consisting of supercoiled DNA strands and DNA-bound proteins, \cite{1,6,9,10} as illustrated in Fig. 1 (see below for details). We complement our simulation, limited to a relatively-short chain, with a free-energy approach, which is valid in the long chain-limit. Our simulation results suggest that crowding effects are strong enough to condense the chain in a cylindrical space, similarly to what was observed for the *E. coli* chromosome.\footnote{Dextran is considered a polymeric crowder.} They also indicate that the compaction is steep but continuous. While this is consistent with our theoretical prediction, its seeming contradiction with the phase coexistence observed for the *E. coli* chromosomes\footnote{Recent studies indicate an abrupt compaction of DNA and its seeming contradiction with the phase coexistence observed for the *E. coli* chromosomes.} can be attributed to the structural heterogeneity and other biological details. Indeed, recent studies that an abrupt compaction of DNA molecules by dextran in a cylindrical tube but a continuous compaction in a slit-like or unconfined space.\footnote{In a recent study, it is considered that the compaction is steep but continuous.}

In our simulations, we have to specify interactions between various pairs, monomer-monomer, monomer-crowder, crowder-crowder pairs, along with interactions of monomers or crowders with the cylindrical wall. In our simulation, the cylindrical wall is formed by imaginary spherical particles, which are of the same kind as monomers. Let \( r \) be the center-to-center distance between two particles. They interact with each other through the fully-repulsive Weeks-Chandler-Anderson (WCA) potential,\footnote{Molecular crowding influences other processes such as DNA reaction and diffusion, bimolecular aggregation, translation, and cell growth.} given by

\[
U_{\text{WCA}}(r) = \left\{ \begin{array}{ll}
4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r} \right)^{12} - \left( \frac{\sigma_{ij}}{r} \right)^{6} + \frac{1}{4} \right] & \hbox{for } r < 2^{1/6}\sigma_{ij}, \\
0 & \hbox{otherwise}
\end{array} \right.
\]  

\hfill (1)

Here the subscripts \( i, j = 1, 2, 3 \) are used to refer to monomers, crowders, and wall-forming particles, respectively: \( \sigma_1 = \sigma_{11} = \sigma = a \) (monomer size), \( \sigma_2 = \sigma_{22} = a_c \) (crowder size), and \( \sigma_{12} = \sigma_{21} = (\sigma_1 + \sigma_2)/2 \). Finally, \( \varepsilon_{ij} \) measures the strength of \( U_{\text{WCA}} \) for various interaction pairs.

Chain connectivity between two consecutive monomers is insured by the finitely-extensible non-linear elastic (FENE) potential described by\footnote{The chain stretches, which would otherwise remain condensed, leading to a reentrant-like transition.}

\[
U_{\text{FENE}}(r) = -\frac{1}{2} k r_0^2 \ln \left[ 1 - \left( \frac{r}{r_0} \right)^2 \right],
\]  

\hfill (2)

where \( k = 30.0\varepsilon_{11}/\sigma \) and \( r_0 = 1.5\sigma \).
**Fig. 1** (Left) Minimalist model of the nucleoid: beads on a string in a crowded and confined space. Note that in our simulation the cylinder is much longer than it appears in this illustration; it is about three times the equilibrium length of the chain $R_0$ in the absence of crowders. The entire system, excluding the chain trapped in the volume $V$, is then periodically reproduced. Thus it better mimics the micro-channel experimental setting than *E. coli* cells. The chain consists of $N$ monomers or beads of diameter $a$ each; the crowding particles are hard spheres of diameter $a_c$ each. The cylinder diameter $D$ is defined as the maximum distance between the centers of two monomers aligned vertically, as shown in the top left corner. The closest center-to-center distance between two spheres (monomers or crowders) sets naturally the parameter $a_{ij}$ that describes their interaction range (one of them is shown in the bottom panel). Each monomer in our work may represent a structural unit in the bacterial chromosome.16 (Right) Depletion forces induced by crowding agents: by overlapping the depletion zones (dashed circles) around two neighbouring monomers, the system increases the entropy of crowders. The resulting entropic force is known as depletion force. If $V_{\text{overlap}}$ is the volume of the overlapped (shaded) region, it can be expressed as a function of the center-to-center distance of the two monomers denoted as $r$: $V_{\text{overlap}}(r) = \frac{4}{3} \pi (a + a_c - r)^2 (a + a_c + r/2)^3$; it reaches its maximum at $r = a$, given by

$$V_{\text{overlap}}(r = a) = \frac{4}{3} \pi a_c^2 (3a/2 + a_c) \equiv \Delta V_{\text{max}}.$$  

Let $P_{\text{ex}}$ be the osmotic pressure of crowders in a monomer-free region, then the depletion force between two monomers is given by $f_{\text{dep}} \approx P_{\text{ex}} \times \frac{\partial V_{\text{overlap}}}{\partial r}$. Of particular interest is the maximum free energy gain of two monomers when brought in contact: $\Delta F_{\text{max}} \approx p_a \Delta V_{\text{max}} \sim p_a a_c^2 (3a/2 + a_c) \sim \phi (1 + 3a/2a_c).$
Our simulation mimics the experimental setting\(^{16}\) better than the nucleoid in that the cylinder has open ends. This will allow us to examine how crowders condense the chain from \(R_0\) the equilibrium length of the confined chain in the absence of crowders, which is much longer than the nucleoid length. In our simulation, the length of the cylinder \(L\) is three times \(R_0\); periodic boundary conditions are then imposed on crowders at cylinder ends.

For a similar reason, we initially set \(\varepsilon_{13} = \varepsilon_{31} = 5.0\) (for the interaction between the cylinder wall and monomers), so as to prevent the monomer adhesion to the cylinder wall, while setting all other \(\varepsilon_{ij}\) to 1.0 (in units of \(\varepsilon_{11} = \varepsilon\)). In micro-channels, chromosome adhesion to the channel wall was chemically discouraged.\(^{16}\) A similar physical picture was used in the concentric-shell model of the nucleoid, where the chromosome is mostly confined inside the inner cylinder.\(^{30,31}\) Finally we also examined how the choice of \(\varepsilon_{13} = \varepsilon_{31}\) is implicated in chain compaction or chain adsorption. To this end, we varied it from 1.0 to 10.0.

The equation of motion is integrated using the velocity-Verlet algorithm with a time step \(0.002\tau_\phi\), where \(\tau_\phi = \sigma\sqrt{m/\varepsilon}\) (\(m\) is the bead mass). We keep the system at a constant temperature given by \(T = 1.0\varepsilon/k_B\) via a Langevin thermostat with a damping constant \(\gamma = 0.1\tau_\phi^{-1}\) (see Refs.\(^{32,33}\) and references therein).\(^{3,6}\) Here and below, \(k_B\) is the Boltzmann constant and \(T\) the temperature. In our actual simulations, the simulation package LAMMPS is used.\(^{34}\)

Here we chose the farthest distance as the chain size\(^{35}\) and measured it every 1000 integration steps after the chain equilibrated. (The farthest distance suffers less from effects of finite-chain sizes\(^{35}\) and is more free from numerical artifacts.) The ensemble average \(<...>\) was taken over more than 20 independent simulations and over about \(5 \times 10^7\) integration steps for each simulation. The ensemble-average of the chain size was obtained as a function of the volume fraction of crowding agents.

For our simulation, we chose the number of monomers \(N = 80\) and the number of crowding particles up to 100000, depending on the volume fraction of crowding particles \(\phi_c\). The size (diameter) of crowding particles were \(a_c = 0.2, 0.3, 0.4, 0.6, 0.8\) (in units of \(\sigma = a\)).

### 3 Results

#### 3.1 Chain compaction: crowder’s sizes and poly(bi)-dispersity

First, we have examined how crowding particles condense a confined chain from its equilibrium length \(R_0 \approx N a (D/a)^{-2/3}\) (in the absence of crowding particles).\(^{37,38}\) In our entire simulations, the chain consists of \(N = 80\) monomers, immersed in the “sea” of crowders, as illustrated in Fig. 1; recall our simulation setting resembles the experimental setting\(^{16}\) (see Sec. 2).

Fig. 2 displays our results for chain compaction for various parameter choices, for a mono-disperse (a) and bi-disperse case (b); here \(R/R_0\) is plotted as a function of the volume fraction of crowders \(\phi_c\) (Left) and their number density \(\rho_c\) (Right). Note that unless otherwise stated lengths are measured in units of \(\sigma = a\), as is the case for Figs. 2-6.

As shown in the graph on the left in Fig. 2(a), the polymer chain shrinks in size as the volume fraction of crowding agents \(\phi_c\) increases as long as \(a_c < 0.6a\). They induce entropic attractions, often referred to as depletion forces, between monomers that would otherwise repel each other and swell the chain (see Ref.\(^{13-15}\) for the nature of these forces in a more general context as well as Ref.\(^{39,40}\) for chain compaction in an uncon fined space or in a poor solvent). With smaller crowders, the chain is compacted at a smaller volume fraction. This also explains why too large ones fail to condense the chain (shown in grey); they are ineffective. On the other hand, on a density basis, larger ones condense the chain more effectively except for \(a_c = 0.6a, 0.8a\). For too large \(a_c\), the desired value of \(\rho_c\) for compaction cannot be reached.

These findings are in qualitative agreement with the physical picture illustrated on the right in Fig. 1. Each monomer is surrounded with a ‘depletion layer,’ described by a dashed line, in which the center of crowders is not accessible.\(^{15}\) Overlapping of depletion layers allows the surrounding crowders to explore a larger space. The resulting entropic (depletion) force is related to \(V_{\text{overlap}}\), the volume of the overlapped (shaded) region between two monomers. If \(r\) is the center-to-center distance of two adjacent monomers, \(V_{\text{overlap}}(r) = \frac{\pi}{6}(a + a_c - r)^2(a + a_c + r/2)\) with its maximum given by \(\Pi_c\) be the osmotic pressure of crowders in a monomer-free region, then the depletion force between two monomers can be obtained approximately as \(f_{\text{dep}} \approx \Pi_c \times \partial V_{\text{overlap}}/\partial r\). Of particular interest is the maximum free energy gain of two monomers, which is reached when brought in contact,

\[
\Delta F_{\text{max}} \approx \rho_c a_c^2 (3a/2 + a_c) \phi_c (1 + 3a/2a_c).
\]  

Note that this is an approximation, since \(\Delta V_{\text{max}}\) is obtained for two monomers while others are ignored; at best, \(\Delta F_{\text{max}}\) is correct at the level of two-body interactions and at a low density of crowders. As the density of crowders increases, higher-order terms (e.g., \(\rho_c^2\)) will have to be included (see Eq. 8). Also two adjacent monomers do not have to be in contact, as assumed for the quantity \(\Delta V_{\text{max}}\).
Fig. 2 Collapse of a flexible chain (N = 80) by crowding agents in a cylindrical space as a function of the volume fraction $\phi_c$ (Left) and the density $\rho_c$ (Right) of crowding particles for the mono-disperse (a) and poly-disperse case (b). (a) As $\phi_c$ or $\rho_c$ increases, the confined chain shrinks in size. Here, $R/R_0$ is the reduced chain size with $R_0$ the equilibrium size in the absence of crowding particles. The chain size reaches a minimum at a certain value of $\phi_c$ or $\rho_c$. The little increase of $R$ beyond this can be attributed to cylinder-wall layering of crowders, which effectively reduces the cylinder diameter and thus expands the chain. A few curves for $a_c = 0.3$ corresponding to different $D$ values (green) tend to collapse onto each other. This implies that cylindrical confinement enters into the picture through $R_0$. The dotted line was obtained using a free energy approach (see Subsec. 3.4). (b) In the presence of a mixture of small ($a_c = 0.2$) and large ($a_c = 0.4$) crowders, chain compaction appears to interpolate the two corresponding mono-disperse cases, each containing one kind of crowder (see Fig. 4 for details). As a result, the polydispersity of crowders does not alter chain compaction qualitatively.
Despite its limitation, the relation in Eq. 3 appears to explain the general trend seen in our observation in Fig. 2: on the basis of \( \phi_c \), smaller particles are more effective crowders, while on a density basis, the opposite is true. A more complete analysis is presented below (see Fig. 4).

Chain swelling beyond the complete compaction (Fig. 2) is a simulation artifact. In our simulation, the initial conformation was chosen to be helically elongated. At sufficiently high \( \phi_c \), which is larger than needed for the full compaction, the chain got trapped in a local minimum. This makes the global chain equilibrium time longer than typical simulation times. A similar non-monotonic dependence was observed in chain compaction by crowders in bulk.\(^{23}\) This is, however, distinct from the non-monotonic dependence of chain sizes on \( \phi_c \) observed in an earlier study, in which crowders were implicitly included.\(^{41}\) As pointed out,\(^{32}\) this non-monotonicity can be attributed to the implicit presence of crowders through theoretically-obtained depletion forces. In our study and in Refs.\(^{23,42}\) explicit crowders were used.

Chain compaction as shown in Figs. 2 and 4 appears to be a continuous transition. Because of finite-chain lengths, however, this is not so conclusive. To further clarify this, we have used a theoretical approach and presented it in Subsec. 3.4. As described by the dashed curve in the graph on the right in Fig. 2(a), our theoretical result, in excellent agreement with data, indicates a continuous transition, similarly to the theoretical analysis in Ref.\(^{16}\) See subsec. 3.5 for more detailed discussions.

Fig. 3 shows our results for the probability distribution (Left) and the variance (Right) of chain sizes, denoted as \( P(R) \) and \( \sigma_R^2 \), respectively; on the right graph, chain compaction (left axis) is contrasted with the variance \( \sigma_R^2 \) (right axis), as a function of \( \phi_c \); the width of \( P(R) \) determines \( \sigma_R^2 \). The effective spring constant of the chain \( k_{\text{eff}} \) is related to \( \sigma_R^2 \) as \( k_{\text{eff}} \sim 1/\sigma_R^2 \). This graph suggests that chain compaction can “stiffen” the chain up to about seventy fold, as evidenced by much reduced \( \sigma_R^2 \) (or much increased \( k_{\text{eff}} \)) for large \( \phi_c \).

So far, we have focused our discussion on a mono-disperse case, i.e., all crowding particles with the same size. To mimic the cellular environment more realistically, we have also considered a mixture of small \( (a_c = 0.2) \) and large \( (a_c = 0.4) \) crowding particles with their relative volume fraction ranging from \( (20\% : 80\%) \) to \( (80\% : 20\%) \). Fig. 2(b) shows our results for this poly-disperse case, which appears to interpolate the two respective mono-disperse cases; poly-disperity does not change chain compaction qualitatively.
Fig. 4 Interplay between crowder size and density for the mono-disperse (Left) and poly-disperse cases (Right). (Note here that lengths are given in units of \(a_c\).) The size of a flexible chain (\(N = 80\)) in a cylindrical space is displayed as a function of the rescaled variable \(a^2_c \rho_c\) for the mono-disperse (Left) or \(\Sigma a^2_c \rho_c\) for the poly-disperse case (Right). (Left) When plotted against \(a^2_c \rho_c\), the curves corresponding to different values of \(a_c\) collapse onto each other. As a result, doubling \(a_c\) is equivalent to increasing \(\rho_c\) fourfold. For the polydisperse case (on the right), we use a mixture of small \((a_c = 0.2)\) and large \((a_c = 0.4)\) crowders with a varying range of their volume fractions \\{\(80\%:20\%\),\(70\%:30\%),\(60\%:40\%),\(50\%:50\%),\(40\%:60\%\)\} are the corresponding number fractions, respectively. Here, \(\Sigma a^2_c \rho_c = a^2_{\text{small}} \rho_{\text{small}} + a^2_{\text{large}} \rho_{\text{large}}\), e.g., \(0.97 a^2_{\text{small}} \rho_{\text{small}} + 0.03 a^2_{\text{large}} \rho_{\text{large}}\) for the volume-fraction mixture \((80\%:20\%)\). Our results on the right suggest that the effect of polydispersity can be mimicked by adjusting the size of crowders in a homogeneous-crowder system, according to our rescaling scheme \(\Sigma a^2_c \rho_c = a^2_{\text{small}} \rho_{\text{small}} + a^2_{\text{large}} \rho_{\text{large}}\).
How are $a_c$ and $\rho_c$ intertwined? Fig. 4 shows the interplay between crowder size $a_c$ and density $\rho_c$ for the mono-disperse (Left) and poly(dis)perse cases (Right). The size of a flexible chain ($N = 80$) in a cylindrical space is displayed as a function of the rescaled variable $a_c^2 \rho_c$ for the mono-disperse (Left) or $\sum a_c^2 \rho_c$ for the poly-disperse case (Right), where the sum is to be carried out over two types of crowders:

$$\sum a_c^2 \rho_c = a_{\text{small}}^2 \rho_{\text{small}} + a_{\text{large}}^2 \rho_{\text{large}}.$$  \hspace{1cm} (4)

(Here and below, for simplicity, $a_c$ is given in units of $a$, especially when it refers to simulation results.) (Left) When plotted against $a_c^2 \rho_c$, the curves corresponding to different values of $a_c$ collapse onto each other. As a result, doubling $a_c$ is equivalent to increasing $\rho_c$ fourfold.

For the poly-disperse case (on the right), we use a mixture of small ($a_c = 0.2$) and large ($a_c = 0.4$) crowders with a varying range of their volume fractions $\{(80\%:20\%),(20\%:80\%); \{(97\%:3\%), (67\%:33\%)}\}$ are the corresponding number fractions. Let $x$ be the number fraction of small crowders, then Eq. 4 can be rewritten as

$$\sum a_c^2 \rho_c = \left[x a_{\text{small}}^2 + (1-x) a_{\text{large}}^2 \right] \rho_c^{\text{total}}.$$  \hspace{1cm} (5)

where $\rho_c^{\text{total}} = \rho_{\text{small}} + \rho_{\text{large}}$ and $x = \rho_{\text{small}}/\rho_c^{\text{total}}$. This relation leads to $0.97 a_{\text{small}}^2 + 0.03 a_{\text{large}}^2 \rho_c^{\text{total}}$ for the volume-fraction mixture (80\%:20\%). Our results on the right in Fig. 4 suggest that the effect of poly-disperity can be correctly mimicked by adjusting the size of crowders in a homogeneous-crowder system, according to our rescaling scheme in Eqs. 4 or 5.

While it is not entirely clear why $a_c^2$ or $\left[x a_{\text{small}}^2 + (1-x) a_{\text{large}}^2 \right]$ is the correct rescaling factor, Eq. 3 offers some insight though not complete. When we used $a_c^2 (3a_c^2/2 + a_c)$ from Eq. 3 as a rescaling factor for the mono-disperse case, the collapse between different compaction curves was somewhat worse than in the results in Fig. 4 (data not shown). Considering the limitations of Eq. 3 as discussed earlier, this is understandable.

Our findings in Fig. 4 will be useful for interpreting experimental data and for making predictions, especially for a poly-disperse case. In particular, they enable us to define an “average” crowder size $\bar{a}_c$ and the density of average crowers $\bar{\rho}_c$, as illustrated on the top panel in Fig. 4:

$$a_c^2 \bar{\rho}_c = \sum a_c^2 \rho_c = a_{\text{small}}^2 \rho_{\text{small}} + a_{\text{large}}^2 \rho_{\text{large}}.$$  \hspace{1cm} (6)

Note here that what this relation determines is the combination $a_c^2 \bar{\rho}_c$. This means that the corresponding mono-disperse case is not uniquely determined. One possibility is to use the same number of crowders in the mono-disperse case: $\bar{\rho}_c = \rho_{\text{small}} + \rho_{\text{large}}$. Then Eq. 6 becomes

$$a^2 = x a_{\text{small}}^2 + (1-x) a_{\text{large}}^2.$$  \hspace{1cm} (7)
(Recall that $x$ is the number fraction of small crowders.) Extended analysis along this line will be useful for making a quantitative sense of cytoplasmic crowders, which present a poly-disperse system.\textsuperscript{45}

### 3.2 Phase separations

We have also considered how the system phase-separates into crowder-rich and chain-rich phases. Unlike a corresponding system in an unconfined space, the phase separation in this case will be anisotropic. Accordingly, we have calculated the spatial distribution of monomers and crowding particles along the long symmetry axis of the cylinder as well as in the radial direction, denoted as \( \rho_c \) and \( \rho_r \), respectively; for \( \rho_c \), the center of mass of a chain is assumed to be located at \( z = 0 \).

Fig. 5 shows our results for \( \rho_c \) (Left) and \( \rho_r \) (Right) for the poly-disperse case \( a_c = 0.2 : 0.4 (40\% : 60\%) \), corresponding to the green line in Fig. 2(b) \( (N = 80 \text{ and } D = 6) \). The rescaling factor \( \rho_{\text{ref}} \) used for the left graph is chosen to be \( \rho_c(z = \infty) \) for crowders and \( \rho_c(z = 0) \) for the chain. (Here \( \rho_{\text{ref}} \) for \( a_c = 0.2 \) is defined independently of that for \( a_c = 0.4 \).) The introduction of \( \rho_{\text{ref}} \) is merely to enhance the visibility of the graph. On the other hand, \( \rho_r \) on the right is a normalized one: \( \int \rho_r 2\pi rdr = 1 \), which is satisfied separately by small and large crowders. For sufficiently large \( \phi_c \) (all curves displayed except for \( \phi_c = 0.155 \)), crowders are effectively depleted from the chain-rich phase. But the boundary between the two phases is smooth somewhat distinct from a sharp one assumed in a theoretical approach (see the Supplementary Information of Ref.\textsuperscript{16} and references therein).

The graph on the right in Fig. 5 shows how crowders and monomers are spatially distributed along the radial direction. In contrast to \( \rho_c \), the radial distribution \( \rho_r \), especially for monomers, does not appear to be sensitive to the degree of chain compaction. The main effect is to enhance the depletion of monomers from the cylindrical wall (see Ref.\textsuperscript{22} for a similar observation with DNA). However, this effect appears to be minor in our flexible-chain case, compared to what was seen with DNA molecules. This means that flexible-chain compaction occurs mainly along the long axis of the cylinder. This justifies the (quasi) one-dimensional approach to chain compaction in a cylindrical space in Ref.\textsuperscript{16} and in subsection 3.4.\textsuperscript{34}

Because of the wall-monomer repulsion, \( \rho_r \) for the chain is maximized at \( r = 0 \), similarly to the corresponding case without crowders.\textsuperscript{33,35} The opposite is expected for the crowders. Even for \( \rho_c = 0.155 \), which is not sufficient to compact the chain, cylinder-wall layering of crowders is apparent, similarly to the layering of monomers in the absence of crowders.\textsuperscript{35} Also varying the size of crowders does not alter \( \rho_c \) and \( \rho_r \) qualitatively.

### 3.3 Chain compaction vs. adsorption

So far, we have used \( \epsilon_{13} = 5.0 \), the strength for the interaction between monomers and the cylinder wall, so as to mimic the experimental setting in Ref.\textsuperscript{16} where the cylinder wall was passivated with PLL-g-PEG [poly(L-lysine)-g-poly(ethylene glycol)]. As a result, chain adsorption was discouraged. This appears to be consistent with the picture of bacterial chromosomes as self-adherent or coherently-shaped objects rather than cell-membrane sticking ones.\textsuperscript{43,44} However, it is not clear if this is a deceptive parallel, since the local organization of chromosomes involves various nucleoid-associated proteins. To further clarify the role of depletion forces in organizing confined polymers, it will be useful to examine chain compaction with varying \( \epsilon_{13} \).

Under different conditions, crowders can induce depletion forces between a monomer and the cylindrical wall, possibly leading to chain adsorption. To understand the interplay between chain compaction and adsorption, we have examined the consequence of varying \( \epsilon_{13} \) and plotted our results in Fig. 6. We have chosen the parameter so that the chain is collapsed for large \( \epsilon_{13} \) values, including \( \epsilon_{13} = 5.0 \) used for the previous results in Figs. 2 & 4 as well as in Fig. 5, assuming that the volume fraction of crowders is sufficiently large. The results in Figs. 6(a) & (b) remain roughly insensitive to \( \epsilon_{13} \), as long as \( \epsilon_{13} \geq 2.0 \) (a) or \( \epsilon_{13} \geq 3.0 \) (b). For \( \epsilon_{13} = 1.0 \), the chain is elongated along the long axis of the cylinder as shown in (a), similarly to what is observed for sufficiently small \( \phi_c \) and \( \epsilon_{13} = 5.0 \). In contrast to the latter case, the resulting \( \rho_r \) in (b) is single-peaked at \( r \approx D/2 \) (\( \int \rho_r 2\pi rdr = 1 \)). This is reminiscent of strong chain adsorption onto the cylinder wall; most of monomers are trapped in a thin layer of thickness comparable to \( a_c \) at \( r \approx D/2 \) (see (b)). The crowding particles induce depletion attractions between monomers and the wall, which turn out to be stronger than the attraction between monomers.

For \( \epsilon_{13} = 2.0 \), the chain is both compacted and adsorbed onto the wall; the tendencies for adsorption and compaction are comparable. This is responsible for the oscillatory behavior of \( \rho_r \) for \( \epsilon_{13} = 2.0 \) in Fig. 6(b). Imagine increasing \( \epsilon_{13} \) from \( \epsilon_{13} = 1.0 \) (complete adsorption), thus reducing the adsorption tendency. Some of monomers become more loosely bound and leave the adsorption layer, forming a layer right next to the adsorption later. Depending on the balance between adsorption and compaction, multiple layers can form, giving rise to an oscillatory behavior of \( \rho_r \) as shown in (b).

Chain adsorption can alter chain conformation and thus \( R \). The graph in Fig. 6(c) shows how chain adsorption and chain compaction are intertwined. For \( \epsilon_{13} = 1.0 \), a reentrant-like transition occurs. Molecular crowding condenses the confined chain initially for small \( \phi_c \) but rather stretches it in some intermediate range of \( \phi_c \). (See Refs.\textsuperscript{45,46} for a similar but somewhat distinct reentrant coil-globule-coil transition of a poly-
Fig. 6 Interplay between chain compaction and adsorption: how to choose $\varepsilon_{13}$, i.e., the interaction strength between monomers and the cylinder wall. (Note here that lengths are given in units of $a$.) The graphs in (a) and (b) display $\rho_z$ or $\rho_r$, respectively, for a wide range of $\varepsilon_{13}$; $\rho_r$ is normalized so that $\int \rho_r 2\pi r \,dr = 1$. Other parameters have been chosen so that the chain is collapsed for large $\varepsilon_{13}$ values, including $\varepsilon_{13} = 5.0$ used for the previous results in Figs. 2 and 4 as well as in Fig. 5. The results remain roughly insensitive to $\varepsilon_{13}$ as long as $\varepsilon_{13} \geq 2.0$ (Left) or $\varepsilon_{13} \geq 3.0$ (Right). For $\varepsilon_{13} = 1.0$, the chain is elongated along the long axis of the cylinder, similarly to what is observed for sufficiently small $\phi_c$ and $\varepsilon_{13} = 5.0$; on the other hand, the resulting $\rho_r$ is single-peaked at $r \approx D/2$. This finding can be attributed to chain adsorption onto the cylinder wall. The crowding particles induce stronger attractions between monomers and the wall. For $\varepsilon_{13} = 2.0$, on the other hand, the chain is both compacted and adsorbed onto the wall. For larger $\varepsilon_{13}$, however, adsorption is discouraged. The graph in (c) summarizes the effect of adsorption on chain compaction. For $\varepsilon_{13} = 1.0$, a reentrant-like transition occurs: the confined chain initially shrinks in size as $\phi_c$ increases from 0, stretches with increasing $\phi_c$ in its intermediate range, and eventually becomes more compact, but not as effectively as in other cases ($\varepsilon_{13} = 2.0, 5.0$). This observation is correlated with our results in (a) & (b). For $\varepsilon_{13} = 2, 3, \ldots$, adsorption will not complicate chain compaction (see (a)), but for $\varepsilon_{13} = 1.0$, the competition between compaction and adsorption leads to a reentrant-like transition. In the intermediate-$\phi_c$ range, i.e., $0.2 < \phi_c < 0.3$, chain adsorption (see (b)) promotes chain stretching.
mer chain.) For large $\phi$, the chain becomes more compact with increasing $\phi$ but not as effectively as in other cases ($\varepsilon_{13} = 2.0, 5.0$). This observation can be understood in light of our results in Figs. 6(a) & (b). For $\varepsilon_{13} = 2.3, ...$, the interdependence between adsorption and compaction is insignificant (see (a)). For $\varepsilon_{13} = 1.0$, however, crowders can induce chain adsorption (see (b)), promoting chain stretching in the intermediate-$\phi$ range, i.e., $0.2 < \phi < 0.3$.

3.4 Free energy approach

As a fitting model, here we present a free-energy approach, in which the chain size $R$ can be obtained by free-energy minimization. Using this, we fit our simulation data for $R$. Let $V$ be the “enveloping” volume of the chain, inside which the chain is enclosed possibly together with crowding agents (see the dashed line in Fig. 1). As the chain is compacted, $V$ decreases. Let $m$ be the number of crowders within $V$. If we ignore boundary effects, the total free energy of the subsystem enclosed within $V$ (or the chain-rich phase) is given by

$$F_{\text{total}} = \frac{m \ln m}{V} \left( -m + B_2 \frac{m^2}{V} + \frac{5}{8} B_2^2 \frac{m^3}{V^2} + A \frac{R_0^2}{R} + \frac{R^2}{2R_0} \right) + N \alpha c^6 + F_{\text{dep}}. \quad (8)$$

Various terms and symbols are defined as follows. The underlined term describes the corresponding crowder-only system ($F_{\text{crowd}}$) (see for instance Ref. 47), where $B_2$ is the second virial coefficient given by

$$B_2 = 4 \times \frac{4 \pi}{3} \left( \frac{a_c}{2} \right)^3. \quad (9)$$

The relevance of higher-order terms can be checked a posteriori.† The next term is the (renormalized) Flory free energy of a confined chain in the absence of crowders; in this expression, $A$ is a numerical prefactor, which can be adjusted for the best fit to numerical data. The second last term is a standard three-body interaction energy, which is needed when the chain is much compressed. Unlike the two-body term included in the term containing $A$, which describes blob-blob overlap free energy, this term captures monomer-monomer interactions. This term is needed to avoid a complete collapse of the chain or $R = 0$; the notion of blobs becomes less meaningful.‡

Furthermore, $F_{\text{dep}}$ is the free energy reduction due to the depletion of crowding agents from the chain and is related to

$$F_{\text{crowd}}:$$

$$F_{\text{dep}} = V_{\text{dep}} \cdot \left( -\frac{\partial F_{\text{crowd}}}{\partial V} \right) = V_{\text{dep}} \cdot \Pi_c. \quad (10)$$

Here $V_{\text{dep}}$ is the volume of the depletion zone and $\Pi_c$ is the osmotic pressure of crowders. A standard choice $V_{\text{dep}}$ per monomer for beads on a string is given by

$$V_{\text{dep}} = \frac{4 \pi}{3} \left( \frac{a + a_c}{2} \right)^3 - \Delta V_{\text{max}} = \frac{\pi}{12} a \left( 2a^2 + 6aa_c + 3a_c^2 \right). \quad (11)$$

Note the volume of each monomer is included as in Refs. 9–11.

On the other hand, the free energy of the system outside the volume $V$ is mainly determined by crowders and is similar to the underlined term in Eq. 8 denoted as $F_{\text{crowd}}$, except that $V$ should be replaced by the total volume of the cylinder minus $V$ and $m$ by the total number of crowders minus $m$.

In equilibrium, there will be chemical and mechanical equilibrium across the boundary of the volume $V$. Let $\mu_i$ and $\Pi_i$ be the chemical potential and osmotic pressure of crowders, respectively, where $i = \text{‘in’ or ‘out’}$. In equilibrium,

$$\mu_{\text{in}} = \mu_{\text{out}} \quad \Pi_{\text{in}} = \Pi_{\text{out}} \quad (12)$$

For our analysis, we assumed that $V = R \times \pi(D/2)^2$ and minimized $F_{\text{total}}$ with respect to $R$ and $m_i$ ($i = \text{in or out}$). Also we tried two choices of $V_{\text{dep}}$ per monomer: the one given in Eq. 11 and $V_{\text{dep}} = \frac{4 \pi}{3} \left( \frac{a}{4} \right)^3$, i.e., the volume around the chain inside which crowders are depleted. For some reason, the latter choice leads to a better quantitative agreement with our simulation data. The red dashed line in the graph on the right in Fig. 2(a) was obtained with this choice and $A = 2.84 \times 6$. Note that this value is about six times the one reported in Ref. 48.

† The term containing $m^3$ here is in part to improve the agreement between our theoretical and numerical results. Without this term, a larger value of $A$ has to be used. See the relevant discussion below Eq. 12. Also this is analogous to the three-body term for a polymer chain, i.e., the last second term in Eq. 8.
‡ It is worth mentioning that the choice of this term does not influence the nature of compaction by crowders. This is in sharp contrast to chain collapse in a poor solvent, for which the coefficient of this term dictates the nature of chain collapse (continuous vs. abrupt)40. If we constructed this term based on blob-blob interactions, the $D$ dependence would be different. Together with insensitivity of chain compaction to the numerical prefactor of this term, this suggests that the details of this term are not reflected sensitively in chain compaction in a crowded medium. Also note that this discussion is limited to a flexible chain and does not necessarily contradicts the observation of an abrupt collapse of DNA in a tube.21,22.
On the other hand, when $V_{\text{dep}}$ in Eq. 11 was used instead, we had to use a much larger $A$ value for the best fit to the data. In our theoretical approach, we used step-wise distributions of crowders and monomers in contrast to what our results in Fig. 5 suggest. Some quantitative disagreement is thus expected. This numerical prefactor changes the transition only quantitatively. We thus content ourselves with a qualitative agreement between theory and simulations.

3.5 Chain compaction: continuous or abrupt

Our theoretical analysis, which is relevant for the long-chain limit, suggests that the compaction transition by crowding agents in a cylindrical space is continuous. This finding is consistent with our simulation results in Fig. 2 (as well as in Fig. 3 (left)). It appears that finite-chain size effects would not alter the transition qualitatively. However, it is distinct from the observation that "naked" DNA molecules undergo an abrupt compaction under cylindrical confinement but a continuous compaction in a slit-like and free space.\(^2^1,2^2\) This suggests that the geometry of confined spaces is well reflected in the way crowders collapse chain molecules.

Intriguingly, DNA molecules under slit-like and tube confinement were seen to elongate initially with increasing concentration of crowders, before they were eventually collapsed.\(^2^1,2^2\) To account for this, one can rely on the physical picture that DNA segments are depleted in a (depletion) layer of some thickness on the order of $\ell_p$ from the confining wall, where $\ell_p \approx 500\,\text{Å}$ is the persistence length of DNA.\(^2^1,2^2\) This allows for an easy access of crowders in this depletion layer, effectively increasing the degree of confinement – more so for a larger $\ell_p$. This explains the initial elongation of DNA molecules under confinement, more effectively for the tube geometry; this non-monotonic behavior was viewed as underlining the abrupt transition in tube confinement.\(^2^1,2^2\) It also implies that chain flexibility can delay or even prevents the emergence of this effect, as seen in Fig. 2.

In contrast to the DNA case, the compaction of $E.\ coli$ chromosomes in tube confinement appears to be monotonic.\(^1^6\) In other words, chain-segment depletion from the confining wall, which was seen in the former case, does not appear to be correlated with the observation of coexisting phases in the chromosome experiment, i.e., chromosome-rich and crowder-rich phases,\(^1^6\) which would be nominally interpreted as a signature of abrupt (first-order) phase transitions. Thus, it is not entirely clear whether the phase coexistence arises from chain stiffness or chain heterogeneity.\(^8\) Imagine a chain molecule consisting of two regions parameterized by different values of $A$ (see Eq. 8). At some value of $\phi_c$, it is possible that one region almost full-compacted can coexist with the other in an extended conformation. This, however, does not necessarily mean that the transition is either continuous or abrupt. For further clarifying the nature of compaction, one should include other biological details our model leaves out.

Along this line, it is worth mentioning that the bacterial chromosome resembles a confined chain, as demonstrated in Ref.\(^1^6\); monomers in our study should be interpreted as a coarse-grained model of the structural units of the bacterial chromosome, each consisting of supercoiled DNA strands and DNA-associated proteins\(^1,1^6,1^9,2^0\) (see the illustration in Fig. 1). Beyond this general picture, the details, best characterized for the $E.\ coli$ nucleoid, vary from reference to reference (see Refs.\(^1^6,1^9,2^0\)). For instance, early measurements suggest that the size of each unit is $a_{\text{unit}} = 70 \pm 20\,\text{nm}$ and the number of units falls around $n_{\text{unit}} = 100$.\(^1^9\) In a more recent estimate, $a_{\text{unit}} = 100-400\,\text{nm}$ and $n_{\text{unit}} = 63-284$ per cell (15-65 per chromosome).\(^1^6\) Our choice of $N = 80$ falls in the acceptable range. Also recall that in our simulations we use $a$ (monomer size) as length units for other quantities such as $a_c$ and $D$ (see Sec. 4 for relevant discussions on our choice of $a_c$); on the other hand, we varied $D$ so as to map out a general picture of how the degree of confinement is reflected in chain compaction, as shown in Figs. 2 and 4 (this effect becomes implicit if $R$ is rescaled with $R_0$). Thus a thin semiflexible chain with a long persistence length may not necessarily describe the bacterial chromosome more adequately.

Indeed, a similar theoretical approach based on a flexible-chain model explains some feature of chromosome-compaction experiments, in which $E.\ coli$ chromosomes were condensed by PEG (polyethylene glycol), which is a polymeric crowder like dextran.\(^1^6\) For instance, it produces the volume fraction of crowders required for compaction comparable to the experimental counterpart.

4 Discussions and conclusions

In conclusion, we have examined how a flexible chain with self-avoidance is condensed by crowding particles in a (bacterial-cell-like) cylindrical space. Crowding particles induce entropic (depletion) forces between monomers, which compete with the direct repulsion between monomers. At a sufficiently high volume fraction of crowders, the depletion force is strong enough to collapse the chain, similarly to what was observed for bacterial chromosomes.\(^1^6\) The compaction appears to be rapid but continuous both in our simulation and free energy analysis. To account for the phase coexistence seen in the recent experiment,\(^1^6\) a more realistic model chromosome will be desired, as noted above.

A salient feature of our results is the interplay between the size $a_c$ and density $\rho_c$ of crowders as summarized by

\(^8\) Chain stiffness is not well known for the chromosome, since chromosome-bound proteins can modify the physical property of the DNA. As a result, our bead-spring model in Fig. 1 represents the chromosome better than it may seem at first glance.
the simple relation \( a_c^2 \rho_c \) or \( \sum a_c^2 \rho_c = a_{\text{small}}^2 \rho_{\text{small}} + a_{\text{large}}^2 \rho_{\text{large}} \) for a monodisperse or polydisperse case, respectively, against which chain compaction becomes independent of \( a_c \) or poly(bi)dispersity \( (a_c \text{ given in units of } a) \). According to this, in the monodisperse case, doubling \( a_c \) is equivalent to quadrupling \( \rho_c \). Furthermore, the effect of polydispersity can be adequately mimicked by adjusting \( a_c \) in the corresponding monodisperse case. This finding, experimentally testable, can inspire new experiments. For instance, it will be useful to repeat the recent \textit{E. coli} compaction experiment\(^{16}\), using a poly-disperse mixture of crowders. Because of the coarse-grained nature of our model nucleoid, one has to use due caution to test our results against experiments with the biological object: the nucleoid. For instance, our results suggest that the required volume fraction of crowders for full compaction is \( \phi_c \approx 0.2 \) (or 20%) when \( a_c = 0.2 \) (in units of \( a \)) is chosen. At first glance, this value appears to be in a reasonable agreement with the volume fraction of “free” proteins in the cytoplasmic space (\( 12\%-17\% \)) and that of PEG in \textit{in vitro} experiments (\( 11\%-13\% \)).\(^{5,16}\) However the size of crowders also matters (see Figs. 2 and 4).

Considering that the size of structural units is in the range of \( a \approx 100 \text{ nm} \) (up to 400 nm)\(^{16}\) and the size of proteins in the range of \( a_c \approx 4.6 \text{ nm} \),\(^{1,12}\) \( a_c = 0.046 \) in units of \( a \). On the other hand, \( a_c^2 \rho_c \approx \phi_c / a_c \). Thus (\( \phi_c = 10\%, a_c = 0.046 \)) maps onto (\( \phi_c = 43\%, a_c = 0.2 \)). Taken literally, the cytoplasmic space has at least twice as many proteins as required for chromosome compaction (in addition to other biomolecules); the resulting depletion force can be strong enough to condense the chromosome.

In principle, one can improve the analysis above by correctly weighing various types of crowders in the computation of an average crowder size \( a_c \), assuming that Eq. 6 or Eq. 7 works for a mixture of more than two kinds of crowders: \( \bar{a}_c^2 \rho_c = \sum a_c^2 \rho_c \), where the sum is over all types of crowders. Note here that what this relation determines is the combination \( \bar{a}_c^2 \rho_c \). Use of this rescaling relation for the cytoplasmic proteins would require more complete knowledge about crowder sizes/densities in the cytoplasmic space.

Nevertheless, a physical picture emerging from our considerations is that depletion forces are as tangible as some other forces involved in biomolecular processes: hydrophobic forces responsible for the folding of proteins into their native structure\(^8\) and counterion-induced attractions between DNA strands needed for DNA packing as in viruses.\(^{50}\) Importantly, they are the main player in packing the bacterial chromosome. Indeed, the nature of these forces is as intriguing as that of electrostatic attractions between like-charged molecules.\(^{50}\) To our mind, they are best caricatured as ‘attraction through repulsion’\(^{15}\) (see also Fig. 4 in Ref.\(^{16}\)). New experiments under controlled conditions (e.g., crowder sizes and poly-dispersity) will be useful for further clarifying the nature of these forces and for testing our results.

As more computational power becomes available, one can use a more realistic model of chromosomes such as a semiflexible-chain with crosslinks, interacting with crowders. Importantly, the favorable role of crowders goes beyond single-chromosome packing. It has been recently shown that they play an important role in properly partitioning two daughter chromosomes in a dividing bacterial cell.\(^{51,52}\) Our results reported here, especially for the poly-disperse case, will be useful for further appreciating the role of cytoplasmic crowders (with variable sizes) in organizing and segregating bacterial chromosomes.

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### References


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\(\dagger\) This is a typical size of cytoplasmic globular proteins. This is essentially identical to the known average protein size 4nm (or a radius 2nm).
52 Y. Jung, private communication (2014).
A graphical and textual abstract for the Table of contents entry

Fig. 7 Compaction of a chain molecule by molecular crowding in a confined space: mono-disperse (upper) and poly-disperse cases (lower). The two cases can map onto each other.