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Rapid Conformational Fluctuations in a Model of Methylcellulose

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Abstract

Methylcellulose is a thermoresponsive polymer that undergoes a morphological transition at elevated temperature, forming uniform diameter fibrils. However, the gelation mechanism is still unclear, in particular at higher polymer concentrations. We use Langevin dynamics simulations to investigate a coarse-grained model for methylcellulose that produces collapsed ring-like structures in dilute solution with a radius close to the fibrils observed in experiments. We show that the competition between the dihedral potential and self-attraction causes these collapsed states to undergo a rapid conformational change, which helps the chain to avoid kinetic traps by permitting a transition between collapsed states. If the dihedral potential is removed, the chains do not escape from their collapsed configuration, whereas at high dihedral potentials, the chains cannot stabilize the collapsed state. We provide systematic data on the effect of the dihedral potential in a model of methylcellulose, and discuss the implication of these previously overlooked, rapid conformational fluctuations on the spontaneous formation of high-aspect-ratio fibrils.

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I. INTRODUCTION

Methylcellulose (MC) is a class of biopolymers investigated extensively to develop materials with innovative physico-chemical properties. MC is categorized as safe by the U.S. Food and Drug Administration [1] and used in a wide variety of commercial products for example as a thickener in pharmaceuticals, cosmetics and food products [2, 3]. Depending on the degree of substitution, MC in water can form a hydrogel upon heating. Recent experimental studies [4, 5] on the gel structure reveal a fibrillar morphology with a uniform diameter. Fibril formation cannot be explained solely by established equilibrium concepts such as Flory-Huggins solution theory [6, 7], thus requiring a new model. From a computational standpoint, this is not an easy problem since the fibrils have a relatively large axial extent (\(\sim \mu m\)), but the chemical details of the polysaccharide units must be included in order to capture the intramolecular interactions leading to fibril formation. Huang et al. [8] recently proposed a coarse-grained model of MC, parameterized from atomistic simulations, in which an isolated MC chain spontaneously adopts a ring structure with an outer diameter consistent with the experimental results. However, demonstrating that a single polymer tends to collapse into a ring does not explain why MC forms a fibril with high-aspect ratio instead of being trapped in other “misfolded” shapes, like bundles or hairpins. We show here that this coarse-grained MC model exhibits rapid conformational rearrangements that have been overlooked in previous reports. We propose that such fluctuations are essential to fibril formation, and elucidate why the underlying coarse-grained model produces rapid rearrangement phenomenon.

MC is a cellulose-based polymer with hydroxyl groups (-OH) partially replaced by methoxy moieties (-CH₃) at the C-2, C-3 and/or C-6 positions. Since there is an irregular distribution of substituents along the cellulosic backbone, MC always appears as a heterogeneous polymer and the degree of substitution (DS) defines the average number of methyl groups per anhydroglucose unit. The DS ranges from zero for unsubstituted cellulose to 3 for the fully substituted polymer. The DS also determines the solubility of MC. For example, a DS of 1.64 to 1.92 yields maximum water solubility, a lower DS leads to MCs that are only soluble in caustic solution due to intramolecular hydrogen bonding, and a higher DS produces MCs that are soluble only in organic solvents because of the hydrophobic methyl groups [9].
The gelation mechanism of MC has been studied both experimentally and theoretically. Typically, water-soluble MC exhibits lower critical solution temperature (LCST) phase behavior in aqueous systems \[10–18\]; MC is soluble in water at low temperature, while thermoreversible gelation occurs at elevated temperature accompanied by an increase in optical turbidity \[4, 5, 19\]. Although a body of literature \[4, 5, 10–17, 20–25\] on MC gelation exists, there is no consensus on the relationship between the LCST phase behavior and the gelation process. Many have attributed this behavior to viscoelastic liquid-liquid phase separation \[4, 5, 10–17, 20–23\], where the polymer-rich and polymer-deficient phases are kinetically trapped. In an effort to address this phase-separation hypothesis, some studies have attempted to build a temperature-composition phase diagram \[10, 12, 25\]. Unfortunately, there is no conclusive evidence to clearly show the coexistence and stability curves for the phase diagram, which are crucial for the liquid-liquid phase separation behavior.

Recently, several experimental studies on the structure and physical properties of aqueous MC solutions provided compelling evidence for a heterogeneous fibrillar morphology \[4, 5, 10, 14, 19, 26, 27\], a structural motif common to semiflexible polymers found in living organisms \[28–30\]. This fibrillar morphology and the associated viscoelastic response of these solutions are fundamentally different from what would be expected from a morphology based solely on liquid-liquid phase separation. Understanding the structure of the fibrils as well as their mechanical properties could guide efforts in the design of new and useful materials. Lott et al. \[4, 5\] quantified the fibrillar structure of aqueous MC gels with a combination of (real space) cryogenic transmission electron microscopy (cryo-TEM) and (reciprocal space) small-angle neutron scattering (SANS) techniques. Fibrillar dimensions of 14 ± 1 nm were obtained by fitting the SANS data with a model based on the form factor for flexible cylinders with a polydisperse radius. Surprisingly, this diameter is independent of mass fraction and molecular weight \(M_w\) within the range of 0.01% to 3.79% and 49 to 530 kg/mol, respectively. McAllister et al. \[26\] further investigated the thermodynamics of aqueous MC solutions. They found that the growth of the fibrils cannot be explained solely using classical Flory-Huggins solution theory \[2\], and they proposed that MC fibrils reflect a kinetically trapped state that balances thermodynamic equilibrium and local orientational order. While the gelation mechanism is still an open question, these experimental studies provided an unambiguous picture of the fibrillar morphology.

In order to study the gelation mechanism, several computational studies have been con-
ducted to explore how MC chains \[8, 31\] or, more generically, self-attractive semiflexible polymer chains \[34–36\], collapse in various solution conditions. In a particularly relevant work, Kong et al. \[31\] systematically studied the collapse transition of a generic semiflexible polymer model with variable backbone stiffness and self-attraction strength. They constructed a phase diagram of the resulting collapsed states including toruses, globules and bundles. In a subsequent study, Huang et al. \[8\] developed a coarse-grained (CG) model specifically targeting MC chemistry. They first conducted atomistic simulations for 10-mer MC oligomers \[32\] and then used the resulting data to parameterize the force fields appearing in their coarse-grained MC model. With respect to understanding the connection between fibril formation and gelation, it is important to have a chemically realistic model since the sol-gel transition is closely related to the strength of MC self-attractive interactions. With isolated chain simulations, Huang et al. \[8\] found that at 50 °C, MC chains with 600 monomers or more can adopt a ring-like structure in a dilute aqueous system. Importantly, while this ring structure is not a high-aspect ratio fibril, it is plausible that they are precursors to forming MC fibrils. Based on this coarse-grained model \[8\], Ginzburg et al. \[33\] developed a statistical mechanical model for the behavior of multiple MC chains and proposed that the fibril is a one-dimensional stack formed by MC rings.

Although these computational studies \[8, 33\] provide a plausible model for fibril formation in dilute solution, they leave open two important questions that we address here. First, other collapsed states, such as hairpins and folded bundles, could appear as long-lived intermediate states, or even final (metastable) states \[8\]. How these “misfolded” collapsed states are removed and how to prevent these misfolded states from propagating through fibril formation are unanswered questions. Second, although atomistic simulations suggest \[8, 32\] that the hydrophobic interaction, i.e., the non-bonded self-attraction, is the major driving force for MC gelation, the coarse-grained MC model was parameterized from atomistic simulations of 10-mer oligomers. The dihedral potential obtained from this mapping involves four successive monomers, and it is not obvious that the potential obtained from mapping 10-mers will translate to longer chains. Thus, it is important to investigate the sensitivity of the dihedral potential before drawing conclusions regarding MC gelation, as it competes with the non-bonded interactions that drive the formation of collapsed structure.

We show here that significant insights into the gelation mechanism can be obtained through long-time simulations of single MC chains and a model problem of ring association,
taking advantage of the model proposed by Huang et al. [8]. In particular, we identify a
previously overlooked, rapid conformational fluctuation produced by the dihedral potential
that we posit is important for both escaping misfolded states and guiding fibril formation
for multiple chain simulation. We also study the effects on the dihedral potential on the fre-
quency of this rapid conformational change and shape of the collapsed states, demonstrating
that the fluctuations in the collapsed state are governed by the balance between the dihedral
potential and non-bonded interactions. While this coarse-grained model appears to capture
many of the features of the collapse of a single methylcellulose chain, we show that several
important features for methylcellulose gelation remain unanswered and suggest directions
for further model improvements.

II. MODEL AND SIMULATION METHOD

A. Coarse-Grained Model of Methylcellulose

The model and the simulation method are described in detail by Huang et al. [8]. For
completeness, we outline the key details here and recapitulate the various model parameters
in the Supplemental Material [37]. In the simulations described throughout this work, MC is
modeled as a heterogenous polymer with all eight types of monomers, i.e., cellulose monomer,
2-MC, 3-MC, 6-MC, 2,3-MC, 2,6-MC, 3,6-MC and 2,3,6-MC. The order of the monomers
was generated randomly and the composition profile was chosen to match the mole fraction
of the commercial polymer METHOCEL A [9], as tabulated in the Supplemental Material
[37]. Each bead in the coarse-grained (CG) model represents one monomer. The bead-bead
interaction potential,

\[ U = U_{\text{bond}} + U_{\text{angle}} + U_{\text{dihedral}} + U_{\text{nb}} \]  

contains four parts: harmonic bond, angle, dihedral interactions, and nonbonded potentials.
The first three potentials are of bonded types and expressed as

\[ U_{\text{bond}} = \frac{1}{2} K_b (l - l_0)^2 \]  

\[ U_{\text{angle}} = \frac{1}{2} K_\theta (\theta - \theta_0)^2 \]  

and

\[ U_{\text{dihedral}} = K_d [1 + d \cos(n\phi)] \]
Here $K_b$, $K_\theta$, and $K_d$ are the bond, angle and dihedral force constants, respectively, $l_0$ is the equilibrium bond length, and $\theta_0$ is the equilibrium bending angle. In Eq. 4, $d$ and $n$ are the phase constants. Huang et al. [8] performed atomistic simulations of 10-mer homogenous MC oligomers and determined the bonded parameters for the CG model by mapping the radial distribution functions (RDF) from the atomistic model to their CG counterparts. Although there are 8 different homopolymers, the intramolecular atomistic RDFs are similar among all monomer types. We thus use the same set of the bonded parameters for all monomers, and the parameters are summarized in the Supplemental Material [37]. In what follows, we will be particularly interested in $K_d$, which controls the dihedral strength for four consecutive beads on a chain.

The nonbonded interactions have the form of a truncated and shifted Lennard-Jones (LJ) 9-6 potential,

$$U_{nb} = \varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r} \right)^9 - \left( \frac{\sigma_{ij}}{r} \right)^6 \right] + \frac{1}{4} \left( \frac{\sigma_{ij}}{r_{cij}} \right)^6$$

for $r < r_{cij}$ and $U_{nb} = 0$ otherwise, where $i$ and $j$ denote two types of MC monomers. The intermolecular nonbonded interaction parameters among the same type of monomers, i.e., $\sigma_{ii}$, $r_{cii}$ and $\varepsilon_{ii}$, were obtained again by Huang et al. [8] by matching the atomistic simulation of short homogenous oligomers to the CG models. The bead size and cut-off radius, $\sigma$ and $r_c$, depend on the type of monomers and the ambient temperature, while $\varepsilon$ is also an explicit function of contour length. Note that the “effective temperature” is captured by the nonbonded parameters rather than being an explicit function of temperature in the CG simulation. We have tabulated the nonbonded parameters in the Supplemental Material [37] for a representative low temperature (25 °C) and a representative high temperature (50 °C). At 25 °C, MC is soluble in water, while gelation can occur at 50 °C [38]. For the nonbonded interactions between different types of monomers, we used a geometric mixing rule to calculate the $\sigma_{ij}$, $\varepsilon_{ij}$ and $r_{cij}$ in the heterogenous MC chains. The mixing rules are also included for completeness in the Supplemental Material [37].

B. Simulation Method

Following prior work [8], we simulated this coarse-grained model using Langevin dynamics with the velocity-Verlet integrator in the LAMMPS package [39] (ver. Jun 2014) in the
NVE ensemble. Simulations were set up using LJ units with three fundamental scales: \( m \) for unit mass, \( \sigma \) for unit distance, and \( \varepsilon \) for unit energy. We chose the unit mass to be the average molecular weight of all 8 different types of MC monomers, 188 Da. Although different types of monomers have different mass depending on how many hydroxy groups are substituted, the additional complications introduced by accounting for small changes in mass are not consequential within a coarse-grained, Langevin dynamics simulation. We set the unit length to be 0.515 nm, which is the simulated averaged center-of-mass separation of monomers from previous atomistic simulations [8]. The unit energy, \( \varepsilon \) is set to be \( k_B T \) with \( k_B \) being the Boltzmann factor and \( T = 298 \) K. We ran our simulations using a Langevin thermostat at the unit temperature, and the “effective temperature” is captured by the self-attraction force field between MC monomers as described above. A summary of all the dimensionless parameters and their corresponding dimensional parameters are tabulated in the Supplemental Material [37].

In addition to these three fundamental quantities, the damping parameter in the Langevin dynamics algorithm plays an important role in determining the relationship between simulation time scale and real time scale. Although the unit time is not a fundamental unit and can be expressed as \( \tau = (m\sigma^2/\varepsilon)^{1/2} \), its dimensional counterpart can not be calculated directly from the dimensional value of \( m, \sigma \) and \( \varepsilon \) because they contain no information about the solvent. Thus, the damping parameter is specified in time units and is regarded as inversely related to solvent viscosity. We set the damping parameter to be \( 10\tau \), leading to the estimate \( \tau = 0.028 \) ns. This value was reported by Huang et al. [8] and we confirmed it by measuring diffusion coefficients in our simulations at \( T = 25 ^\circ C \).

Unless otherwise specified, we initiated our simulations with random sequences for heterogeneous MC. We simulated 1000-mer MC chains with periodic boundary conditions and a box size length of 600\( \sigma \). The simulation results reported for each value of \( K_d \) include at least three independent trajectories. To study the collapse of the chain above the gelation temperature, the MC chain is first equilibrated at low temperature (25 \( ^\circ \)C) for \( 10^7 \) steps at a time step of \( 5 \times 10^{-4}\tau \), and an instantaneous temperature jump is introduced by switching the nonbonded parameters from 25 \( ^\circ \)C to 50 \( ^\circ \)C. Then the simulation proceeds for at least \( 5 \times 10^7 \) steps with time step of \( 5 \times 10^{-4}\tau \).
C. Data Analysis

We study the rapid conformational fluctuations by monitoring the shape of the MC chain, and we will call this rapid change a “flipping event,” which we discuss later in more detail. Specifically, three quantities are used for structural characterization: the eigenvalues of the gyration tensor \( \lambda \), the radius of gyration \( R_g \), and the relative shape anisotropy \( \kappa^2 \).

The gyration tensor quantifies the second moments of monomer positions on a polymer chain,

\[
S_{mn} = \frac{1}{N} \sum_{i=1}^{N} r_m^{(i)} r_n^{(i)}
\]

where \( r_m^{(i)} \) is the \( m \)-th Cartesian coordinate of the position of the \( i \)-th monomer. The coordinate system has been chosen so that the center of mass lies at the origin. Since the gyration tensor is a symmetric 3×3 matrix, diagonalization produces the principal moments of the gyration tensor, i.e., the squared eigenvalues. The eigenvalues are ordered such that \( \lambda_x^2 \leq \lambda_y^2 \leq \lambda_z^2 \). The eigenvalues of the gyration tensor measure the extensions in the principle axis system, thus give the dimensions of an object.

The principal moments can be combined to give two scalar quantities that describe the shape of the polymer conformation, thereby enabling us to locate the flipping events without the need to consider the detailed configurations of the chain. The squared radius of gyration is the sum of the principle moments of the gyration tensor,

\[
R_g^2 = \lambda_x^2 + \lambda_y^2 + \lambda_z^2.
\]

We found that \( \Delta R_g/R_g \), i.e., the relative difference of radius of gyration between two time frames, is a useful proxy for capturing the conformational change. We chose the time difference for computing \( \Delta R_g \) to be \( 10^5 \) steps, or 50\( \tau \). A peak of \( \Delta R_g/R_g \) signals a sudden conformational change in the polymer size, and thus a potential flipping event. We set the threshold of a flipping event to be \( \Delta R_g/R_g \geq 0.1 \) to screen out typical changes in \( R_g \) due to thermal fluctuations, and we also cluster peaks within 50\( \tau \) to be a single flipping event in order to avoid overcounting large conformational changes immediately following a flipping event that result from the instability of the relatively open chain configuration.

Finally, the relative shape anisotropy [40, 41] is defined as

\[
\kappa^2 = 1 - 3 \frac{\lambda_x^2 \lambda_y^2 + \lambda_y^2 \lambda_z^2 + \lambda_z^2 \lambda_x^2}{(\lambda_x^2 + \lambda_y^2 + \lambda_z^2)^2}.
\]
This shape descriptor reflects both the symmetry and dimensionality of a polymer conformation; the minimal value $\kappa^2 = 0$ indicates a highly symmetric conformation while the maximal value $\kappa^2 = 1$ indicates all beads lying on a line. For a planar symmetric structure, for example a ring conformation, $\kappa^2$ is around $1/4$. \[42\]

III. SINGLE CHAIN RESULTS

A. Flipping Events in an Isolated MC Chain

We first simulated an isolated MC chain with $N = 1000$ at 50 °C, where the self-interactions between MC monomers are strong enough to form collapsed structures in the model of Huang et al. \[8\]. It has been shown by simulation \[8, 33\] that, at elevated temperatures, a single MC chain with 600 monomers or more can form a ring structure. We confirmed this result in our simulations.

Interestingly, we observed that after the chain forms a collapsed structure, large conformation changes can happen within a short period of time that do not correspond to the expected breathing modes of a ring. Figure 1 shows a typical flipping event in a single 1000-mer MC chain. The total simulation time is $3.4 \times 10^4 \tau$, which corresponds to 952 ns. We started the high temperature simulation with an initial configuration generated from a room temperature simulation, as described in the Methods. Because at low temperature the self-attraction is insufficient to collapse the chain, the MC chain is initially a random coil. At $t = 0$, we elevated the temperature and found that the conformation of the MC chain first becomes a collapsed structure at $t = 280$ ns, consistent with previous work \[8, 33\], and undergoes the small shape fluctuations one would expect for a collapsed ring. However, at $t = 592$ ns, as shown in the first snapshot of Fig. 1, the chain adopts a bifocal structure. Within the next 7 ns, the chain goes through a huge conformational change with the whole collapsed structured disturbed. Owing to the self-attraction forces, this loose structure is not stable. After another 7 ns, the chain collapsed back into another relatively tight structure, thus converting from one collapsed conformation to another. We have included the movie of the whole trajectory, along with the rotating views of the three representative snapshots, in the Supplemental Material \[37\]. The dynamics in this particular example are not a special case; flipping happens quite often throughout the course of simulation. We posit that flipping...
FIG. 1. Snapshots of a flipping event of a 1000-mer MC chain at 50 °C. The time lag between each snapshot is 7 ns, corresponding to 250 \( \tau \).

FIG. 2. Radius of gyration and the relative difference of \( R_g \) for a 1000-mer MC chain as a function of simulation time. (a) \( R_g \) versus time for three independent trajectories with 1000 monomers. The three trajectories are of total duration \( 3.16 \times 10^4 \tau \), \( 3.475 \times 10^4 \tau \) and \( 5 \times 10^4 \tau \). Before \( t = 0 \) the chain was equilibrated at room temperature. At \( t = 0 \) the temperature was elevated to 50 °C. (b) \( \Delta R_g/R_g \) versus time for the same three trajectories. An offset of 0.5 on y-axis for each trajectory has been made for clarity. A total of 14, 18 and 25 flipping events are identified for top to bottom trajectories, respectively.

Events are of great importance because they impart an ability to alter rapidly the collapsed structures, which are regarded as local energy minima and represent metastable states.

If we assume that a sudden change of the conformation indicates a flipping event, we can identify the flipping events by monitoring the changes of radius of gyration. Figure 2.
shows how $R_g$ and $\Delta R_g/R_g$ evolve with respect to simulation time for three independent trajectories with $N = 1000$ at $50 \degree C$. Although different trajectories differ in their details, as expected from a stochastic simulation, the overall trends indicate that the chain size and flipping frequencies are robust to the initial conditions and the thermal noise. At the start of the simulation, $R_g$ is relatively high because the MC chain begins in a coiled state. As simulation proceeds, $R_g$ decreases due to the collapse of the MC chain, and reaches a plateau at $t \sim 1 \times 10^4 \tau$. The average value of the plateau for $R_g$ is around 6 nm. After this time, $R_g$ stays in a small range with typical thermal fluctuations. At a few time points, $R_g$ goes through a sudden change, indicating the flipping events. These fluctuations are challenging to identify from $R_g$ alone, so the lower plot of Fig. 2 shows $\Delta R_g/R_g$ with respect to time. This plot clearly shows the location of sudden change of the chain conformation, readily distinguishing the flipping events from more subtle thermal fluctuations. To automate the identification of flipping events, we set the threshold of a flipping event to be above $\Delta R_g/R_g = 0.1$.

Of particular interest are the kinetics of the flipping events. We calculated the time interval between two flipping events by subtracting the time of a flipping event from its successor,

$$\Delta t_n = t_{n+1} - t_n.$$  

Figure 3 shows a histogram of the resulting time interval distribution. Along with the three trajectories in Fig. 2 we added two more trajectories to improve the sampling. The median
FIG. 4. (a) Shape anisotropy $\kappa^2$ and (b) the individual eigenvalues of the gyration tensor $\lambda_i$ versus time for a 1000-mer MC chain. The temperature jumps from 25 °C to 50 °C at the time $t = 0$ (not indicated on the figure). The (red) dots indicate flipping events. (c) Snapshots at (i) 731 ns, (ii) 889 ns and (iii) 1054 ns, which correspond to $2.61 \times 10^4 \tau$, $3.18 \times 10^4 \tau$ and $3.77 \times 10^4 \tau$, respectively. The corresponding time points in panels (a) and (b) are indicated by the dashed vertical lines.

Of the time interval distribution is $950\tau$. Compared to a typical flipping time duration of $250\tau$, as shown in Fig. 1, the flipping event happens quite often and thus is able to alter the chain conformation within a short period of time.

We found that the eigenvalues of the gyration tensor $(\lambda_x, \lambda_y, \lambda_z)$ and the relative shape
anisotropy ($\kappa^2$) are particularly insightful for characterizing the chain configurations before and after flipping events. In Fig. 4(a), we plot part of one trajectory with $N = 1000$ for an isolated MC chain at 50 °C. Similar plots for other trajectories are included in the Supplemental Material [37]. We also show three representative conformation snapshots in Fig. 4(b). During much of the simulation, the shape anisotropy is around 0.25, which indicates a planar symmetric structure. However, we see fluctuations throughout much of the range of $\kappa^2$, ranging from 0.1 and 0.85, indicating other shapes. In the three representative conformations in Fig. 4(b), the first is of a misfolded bifocal structure with some loose “arms.” Because this structure is not stable, and in the process of re-orientating itself, the corresponding value of $\kappa^2$ is part of a large fluctuation at the time indicated by (i) in Fig. 4(a). After several flipping events, the chain conformation adopts a highly anisotropic bundled structure with a relatively high value of $\kappa^2 \approx 0.8$. The last structure shows a return to a ring shape with $\kappa^2$ close to 0.25, indicating a planar symmetric structure. Thus $\kappa^2$ is very helpful in discriminating shapes between bundled and planar configurations, the two most common collapsed structures observed in our simulations.

However, $\kappa^2$ does not discriminate between cases (i) and (iii) in Fig. 4, i.e., between planar symmetric structures with different shapes. Thus, it is useful to consider also the eigenvalues of the associated gyration tensor, which measure the extension in the principle axis system. Some small fluctuations, as well as large jumps, are also observed Fig. 4(a) for the individual eigenvalues. We observed that for the third structure, corresponding to the desired ring structure, $\lambda_y$ and $\lambda_z$ are almost identical. These two eigenvalues indicate the outer diameter of the axisymmetric ring conformation, while the smaller eigenvalue $\lambda_x$ indicates the thickness of the ring. The eigenvalues around (i) are fluctuating substantially, consistent with our discussion of the shape anisotropy. The eigenvalues for (i) are also very different from those for (iii), and this difference allows us to identify the two structures. For (ii), we just have one large eigenvalue ($\lambda_z$) and two smaller eigenvalues ($\lambda_x$ and $\lambda_y$), indicating a bundled structure. Thus, $\kappa^2$ and eigenvalues of the gyration tensor are complementary indicators that together describe the highly structured conformations of the MC chain.

To further explore the effects of the flipping event on the shape of the collapsed states for an isolated MC chain, Fig. 5 presents a ternary plot for the eigenvalues of the gyration tensor using the five trajectories from Fig. 3. The idea is to learn how the dimensions of the conformation are distributed throughout the course of a simulation, as well as to evaluate
FIG. 5. Ternary plot of the rescaled eigenvalues $\left(\lambda / R_g\right)^2$ for 1000-mer MC chains at 50 °C. The eigenvalues are ordered as $\lambda_x \leq \lambda_y \leq \lambda_z$ and satisfy $\lambda_x^2 + \lambda_y^2 + \lambda_z^2 = R_g^2$. Data points are sampled every 50τ. The data correspond to the second half of 5 independent trajectories to remove the effects of the initial configuration and make sure the MC chain has already reached or visited a collapsed state. The 2006 data points are binned with bin size of $\Delta \left(\lambda_x / R_g\right)^2 = 0.01$ and $\Delta \left(\lambda_y / R_g\right)^2 = 0.01$. The colormap shows the probability of observing the combination of eigenvalues in a given bin. A representative MC conformation within the highest probability bin is included.

The likelihood of different collapsed shapes. We plot three rescaled eigenvalues $\left(\lambda / R_g\right)^2$ in the order of $\lambda_x^2 \leq \lambda_y^2 \leq \lambda_z^2$. A striking feature is that the most probable conformation corresponds to $\lambda_x < \lambda_y \approx \lambda_z$; this indicates that most of the simulation time is spent in a ring configuration. Also, the eigenvalues are distributed due to the combined effects of regular thermal fluctuations, flipping events, and the unstable conformation following the flipping events. Overall, Fig. 5 provides a detailed picture of how different shapes are distributed in the course of simulation for isolated 1000-mer MC chains at elevated temperature.

Our discussion is predicated on the assumption that the ring structure is the free energy minimum for an isolated MC chain. This assumption is supported by the probability of observing a ring structure in Fig. 5 and previous work using this MC model [8, 33]. However, as our simulated trajectories are relatively short, it is possible that data such as those in
FIG. 6. Box plot of the time between two flipping events with respect to the strength of the dihedral potential, $K_d$, at 50 °C. The box represents the interquartile range, which contains 50% of the values. The whiskers extend to cover 99.3% of the values. The line across the box is the median value. Each circle shows one data point, with the ones above the whiskers denoting outliers.

Fig. 5 do not completely sample the phase space, especially if there are large barriers between different metastable configurations. Ideally, we could show that the ring state is stable by mapping the present model [8] to a generic model for the collapse of semiflexible chains [31, 43]. Unfortunately, such mapping is frustrated by the number of nonbonded interaction parameters in the model and the use of a 9-6 Lennard-Jones potential [8]. Definitively demonstrating that the ring conformation is the stable state could be achieved by computing the free energy as a function of radius of gyration and shape anisotropy, which is a promising direction for future work on the single-chain MC model.

B. Effect of Dihedral Potential

The results discussed thus far were obtained using the coarse-grained MC model parameters proposed by Huang et al. [8]. We also investigated how these results change as a function of the parameters, and identified the dihedral potential as the most important contributor to the flipping events. Thus, it is illuminating to understand, in a systematic way, the role of the dihedral potential. While arbitrary choices of the dihedral potential will no longer correspond to a coarse-grained MC chain, they allow us to understand the sensitivity of the results to the particular value of $K_d$ appearing in the model by Huang et al. [8].
FIG. 7. Box plots showing the eigenvalues of the gyration tensor ($\lambda_x \leq \lambda_y \leq \lambda_z$) as a function of the dihedral potential $K_d$. The description of the box plots are similar to Fig. 6, except here the outliers are plotted with the (red) cross symbols. (a) $\lambda_x$, (b) $\lambda_y$, (c) $\lambda_z$.

We first quantify the frequency of the flipping events as a function of the strength of the dihedral potential, $K_d$. We chose $K_d$ to be in the range of 0 to 3.0, with a spacing of 0.5. We also attempted to increase $K_d$ further, but found that the calculations were infeasible due to the very small simulation time step required to prevent the simulation from crashing. For each $K_d$, we obtained at least three independent trajectories for at least $2.5 \times 10^4 \tau$. Figure 6 shows a box plot of the distribution of the time interval between flipping events as a function of $K_d$. Separate histograms (similar to Fig. 3) for the time interval distribution for each $K_d$ are included in the Supplemental Material [37]. Note that $K_d = 0$ is not included here because no flipping events were detected. Figure 6 shows that the median of the time between flipping decreases as $K_d$ increases. Furthermore, beyond $K_d = 2.0$, the time between flipping events reaches a plateau of around $1.0 \times 10^3 \tau$. Overall, Fig. 6 clearly shows the likelihood of flipping events strongly depends on $K_d$. Since flipping helps the MC chain explore different free energy minima and different collapsed states, we can conclude that $K_d$ is a key factor in any MC model.

To more deeply understand the effects of $K_d$ and the flipping on the shape of the collapsed states, we created box plots for the eigenvalues for different $K_d$ in Fig. 7. Separate histograms for the eigenvalues for each $K_d$ are available in the Supplemental Material [37]. The first
thing to notice is that, similar to Fig. 5, the median values of the eigenvalues follow the pattern $\lambda_x < \lambda_y \approx \lambda_z$ for all $K_d$. Thus, the polymers are dominantly in a ring conformation, independent of $K_d$. There also seems to be two distinct regimes for $\lambda_x$. For $K_d \leq 1.0$, the median of $\lambda_x$ decreases to slightly below 1 nm. We suspect this is because i) the polymer stays mainly in a ring structure in this regime with few flipping events, and ii) the outer diameter of the ring increases with $K_d$ in this regime, which can be observed from the increase in $\lambda_y$ and $\lambda_z$. Thus the thickness of the ring, embodied in $\lambda_x$, decreases. Furthermore, in this regime, the number of outliers increases as $K_d$ increases due to the increase of the frequency of flipping events. In contrast, for $K_d > 1.0$, the aforementioned increase in the frequency of flipping events leads to both the median and the box size of $\lambda_x$ increasing, corresponding to an increase in the probability of observing unstable loose conformations. Because the flipping events happen more often, $\lambda_x$ values that indicate loose conformations and appear as outliers in box plots for $K_d \leq 1.0$, start to blend into the middle quartiles of the distribution for larger values of $K_d$. As a result, we observe a concomitant decrease of the number of outliers for $\lambda_x$ in the range of $K_d > 1.0$ in Fig. 7. For $\lambda_y$ and $\lambda_z$, both the median and the number of outliers increase as $K_d$ goes up, which is also the result of the increase in the flipping frequency.

IV. IMPLICATIONS FOR FIBRIL ASSEMBLY

Thus far, we have looked into isolated MC chains and shown how flipping events alter the conformation of a single MC chain. However, MC forms high aspect ratio fibrils in experiments, which involve multiple polymers. Although it is challenging to simulate such a large system, we can launch smaller simulations and still gain insights into the fibril formation mechanism. Given the limited number of simulations we can perform for multi-chain systems, our insights should be considered anecdotal, similar to previous work using similar simulations [33].

We thus want to examine, in a simple system, how a flipping event can aid the assembly of a series of smaller rings into a larger tubular structure, even if the initial condition is unlikely to be observed in practice. We return again to the model of Huang et al. [8] where $K_d = 2.0$. We first generate the initial ring conformation by simulating a single 1000-mer MC chain at $K_d = 2.0$. Afterwards, we make four replicas and place the resulting five rings
face-to-face in series with the center-to-center distance along the axis being 5.4 nm, as seen in the first snapshot of Fig. 8. We then launch the simulation for $3 \times 10^7$ steps with a time step of $1 \times 10^{-4}\tau$ under the same elevated temperature, 50 °C.

As the simulation proceeds, the three rings on the top form one tube, while the two rings on the bottom form a second tube. The blue chain, which is on the top of the first tube, then undergoes a flipping event, which allows this chain to stick out several “arms”. Then one of the arms extended out to pass through the hollow space inside the first tube and reached the top face of the second tube. Because the loose conformation of the blue chain is not stable due to the self-attraction, it collapses back into the ring structure. In the meantime it drags the second tube to attach to its tube. After this, the unified proto-fibril relaxes into a more symmetric structure, and then the blue ring goes through another flipping event. The final structure is a single fibril-like structure that has grown in the longitude direction. A movie for this 5-ring simulation is available in the Supplemental Material.

The dynamics in Fig. 8 imply that the flipping events induced by the dihedral potential can facilitate the assembly of distinct rings, which then aid in the formation of the longer fibril structure, in particular to facilitate alignment of the proto-fibrils. We also noticed in Fig. 8 that there are two flipping events in the course of the simulation, and the time interval between these two flipping events is $1430\tau$. This lies within the time interval distribution in Fig. 3 for $K_d = 2.0$, albeit larger than the median. Furthermore, the two flipping events in Fig. 8 both occur for the blue chain, which has one face not attached to other chains. We thus suspect that the energy barrier imposed by neighboring chains plays an important role in the frequency of flipping. If a chain is trapped in the middle of the proto-fibril, it has a lower mobility that prevents escape from its neighbors and flipping into a loose structure. As such, the interior of the tube is stabilized by excluded volume and attractive interactions, while the faces of the tube can flip. The net result is a preference towards axial growth of the fibrils.

While Fig. 8 makes a promising connection between flipping of an individual chain and the dynamics of fibril assembly, there are some limitations of the model and simulation method that attenuate our enthusiasm. First, in the course of simulation, we initiate the simulation by equilibrating the MC chain at 25 °C, and then make an instantaneous temperature jump to 50 °C to form the ring structure. This method ignores the time required for heat transfer, which is a potential but not critical problem. Heat transfer limitations certainly make the
estimation of the time for chain to collapse inaccurate, but the flipping events occur at the higher temperature. Inasmuch as fibril formation takes longer than the time to heat the sample, flipping events could still play a role in the sol-gel transition.

Another important point to consider is the effect of MC concentration. Our single-chain simulations are always in the dilute limit based on the simulation box size. We observed that as we increase the polymer concentration in our simulation and start from a relaxed solution at 25 °C, making a sudden temperature jump to 50 °C leads to the MC chains becoming entangled and trapped in a network, instead of forming rings [33]. Moreover, the branches of these networks do not necessarily have a uniform diameter, contrary to what has been observed in experiments. The issue with polymer density is consistent with what we see in Fig. 8: the frequency of the flipping is reduced due to the more “crowded” environment, thus
making it less likely to cross an energy barrier into another collapsed state. It is definitely of great interest to study a larger system with multiple MC chains at a higher concentration. Unfortunately, the system size and simulation time required to directly simulate gelation are well beyond the computational limits, and suggest the need for a simpler (or softer) model to study gelation.

A way to circumvent this situation and still get an estimate of fibril formation within the present model is to use biased initial configurations. Instead of a completely relaxed-coil structure, the initial configuration can be set as a partially relaxed and a partially ring-like structure. In reality, this initial configuration could correspond to a fast nucleation where part of an MC chain collapses while the rest remains as relaxed intermediate segments or dangling ends. In this way, it might be possible to study the interaction of MC chains at a higher concentration while also preventing the entangled structure. This biased initial configuration could also help to explain the “bridge” or “ghost” structure that connects two successive fibrils along the axial direction, as observed in experiments [4, 5, 19]. In these experiments, MC fibrils consist of collapsed rings that are interlinked by either stacking or wrapping onto the end of an existing fibril or another ring. It is further proposed that the MC gelation is a two-step process [19] beginning with a single or proto-tube nucleus as a precursor, and a secondary nucleation and growth process. This biased partial-coil/partial-relaxed initial configuration could act as an intermediate state in this two-step process to promote our understanding of the secondary nucleation while saving computational effort in the primary nucleation process. Alongside the flipping events, as we described above, this biased initial configuration could also contribute MC free ends that connect between different groups of proto-tubes. These free ends could also rationalize the “bridge” or “ghost” structure, as observed in experiments.

Finally, there is a potential problem using an implicit solvent. Although the gelation in the coarse-grained model is driven by the self-attractive interactions of the MC chain at elevated temperature, in reality, the hydrogen bonding between hydroxy groups on MC and solvent molecules may play an important role in the gelation mechanism. The nature of the interactions between water and MC in the fibril state remains an open question. Moreover, the detailed structure of water in the vicinity of the polymer chains also may be important. While small angle neutron scattering data [3] indicate that the fibrils contain approximately 60% water, neither the latter experiments nor cryo-TEM measurements [4, 5, 19] provide
definitive evidence that the water is located within the core of a fibril. It is possible that
the water and the hydrophilic groups of MC are coordinated, rather than being locally
segregated as predicted by the present model \(^8\). Addressing the details of water-MC co-
ordination in the gel state by simulation is exceedingly difficult, requiring sufficient contour
length to form a fibril while maintaining atomistic resolution. The requisite simulation is
at least an order of magnitude larger than the atomistic simulations \(^3, 2\) used to determine
the coarse-grained parameters used here \(^8\), and likely infeasible. As a result, it is possible
that while the coarse-grained, non-bonded interaction potentials could provide an accurate
representation of that between a pair of very short MC chains, they may not capture the
nature of the interactions in the relatively polymer-dense environment of the fibrils. Elu-
cidating the detailed interactions between water and MC within a fibril may unlock a key
step towards understanding fibril formation.

V. CONCLUSION

Recent experimental studies \(^4, 5\) on the structure of methylcellulose gels revealed a
fibrillar morphology, overturning the prevailing model of physical gelation for these materials.
While the experimental evidence for fibril formation is clear, the detailed gelation mechanism
remains poorly understood. In the present contribution, building on the simulation method
in Huang et al. \(^8\), we showed that the previously overlooked flipping events, characterized
by a sudden chain conformational change, can help a methylcellulose chain to re-orient itself
from one collapsed state to another, as well as facilitating assembly of multiple rings into
stacks. We further studied the methylcellulose chain with a range of dihedral potential
strengths, and found that relatively small changes in the strength of dihedral potential
could have strong effects on the flipping statistics.

We expect that in simulations flipping events will prove particularly important for semi-
flexible self-attractive polymer models, as they help the chain to escape from local energy
traps due to the intramolecular interactions. We also anticipate that this rapid confor-
national change will provide insights into the discrepancy between the high polymer concen-
trations, where fibrils form in experiments, and the low polymer concentration required in
simulations in order to get ring-like structures. However, due to computational limits, we
are unable to directly simulate gelation at a higher concentration. Further progress may
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[37] See Supplemental Material at [URL will be inserted by publisher] for (i) list of simulation parameters (ii) movies of a flipping event and five ring assembly (iii) plots for $K_d=2.0$ (iv) plots for various $K_d$.