Title
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Permalink
https://escholarship.org/uc/item/580730t7

Journal
Physical review. E, Statistical, nonlinear, and soft matter physics, 89(6)

ISSN
1539-3755

Authors
Pandolfi, Ronald J
Edwards, Lauren
Johnston, David
et al.

Publication Date
2014-06-20

DOI
10.1103/physreve.89.062602

Peer reviewed
Designing highly tunable semiflexible filament networks

Ronald J. Pandolfi, Lauren Edwards, David Johnston, Peter Becich, and Linda S. Hirst
University of California, Merced, 5200 North Lake Road, Merced, California 95343, USA
(Received 19 September 2013; revised manuscript received 28 April 2014; published 20 June 2014)

Semi-flexible polymers can generate a range of filamentous networks significantly different in structure from those seen in conventional polymer solutions. Our coarse-grained simulations with an implicit cross-linker potential show that networks of branching bundles, knotted morphologies, and structural chirality can be generated by a generalized approach independent of specific cross-linkers. Network structure depends primarily on filament flexibility and separation, with significant connectivity increase after percolation. Results should guide the design of engineered semiflexible polymers.

DOI: 10.1103/PhysRevE.89.062602 PACS number(s): 61.25.he, 36.20.Ey, 87.10.Tf

I. INTRODUCTION

In biology semiflexible polymers are common, for example F-actin filaments, microtubules, and intermediate filaments form the cell cytoskeleton, cross-linking via the action of a variety of different associated proteins [1]. Recent experimental work has explored the generation of synthetic, peptide based filaments [2], however semiflexible polymers have been little explored for practical materials applications despite their potential to produce unique structures [3–5], light yet rigid gels [6], materials for mesoscale templating [5], biological scaffolds for tissue engineering [7], and drug delivery [8–10].

Semi-flexible polymers have the potential to generate a diverse family of network-based materials. Such materials differ significantly in structure from those seen in polymeric systems formed from molecules approximated by the freely jointed chain. The solution behavior is well known for specific biological examples such as F-actin, microtubules, DNA, etc., under the influence of cross-linking proteins or specific ionic conditions. However, a general picture of phase behavior and the range of accessible structures as a function of flexibility, length, attractive potential, and concentration has not yet emerged, as these parameters are often difficult to tune experimentally. The general principles for assembly and structure tuning we demonstrate open the field for the generation of a wide range of new materials, incorporating semiflexible filaments as the basic unit. There has been limited work dedicated to the formation of materials from stiffer polymers (micron scale persistence length) since most widely used polymer chains are highly flexible. This area of interest has grown recently with a large number of experimental and theoretical studies on the behavior of semiflexible filaments in solution, most biological in origin (i.e., F-actin, DNA, etc.) [3,4,11–29]. Recently Kouwer et al. experimentally demonstrated network formation from a polysaccharide peptide fiber hydrogel with tunable mechanical properties [2]. These materials provide a basis to experimentally investigate functional biomimetic synthetic hydrogels and their applications.

In this paper we explore the range of structures accessible for a semiflexible filament self-assembly. Our coarse-grained model, inspired by models of F-actin networks with explicit cross-linkers, greatly expands the accessible parameter space. Approximating the effect of cross-linkers allows for a more tunable representation of filament attraction and binding. Morphological properties of the networks are quantitatively examined using connectivity analysis, radial pair distribution functions, and a scaling analysis. We demonstrate that hierarchical network structures such as the branching bundle network seen in biological [3,11,12] and synthetic [2] materials do not depend on specific cross-linker interactions, and that these structures can be generated by simple attractive potentials.

Our results reveal that previously observed networks of bundles seen in F-actin systems are not unique to certain cross-linkers but occupy a tunable position in the phase diagram, controlled primarily by filament flexibility and minimum coaxial distance. Filament length is found to have a lesser role, with a minimum length required for network formation. Further modification of filament parameters allows the generation of hierarchically structured networks not seen in flexible polymer systems, such as knotted networks, transverse bundle packing, and examples of structural chirality. Detailing the effects of semiflexible filament parameters on structure and connectivity in this way provides a roadmap for the design of highly tunable hierarchical networks and aids in the discovery of previously unseen structures for novel bioinspired materials.

II. METHODS

A. Simulation model and environment

We can consider two primary mechanisms for semiflexible filament assembly, the action of discrete cross-linkers, or a more general attraction by a local potential (i.e., counterion condensation, van der Waals, etc.). While some cross-linkers may introduce additional geometry to the system (e.g., F-actin and filamin orthogonally bind), many are short and rodlike (e.g., α-actinin, fascin) [1]. Studies have modeled these linkers as charged polyhedra, though this may introduce a set of artifacts, such as cross-linker–cross-linker structuring and same-filament binding [18,19]. Since their principal mechanical function is to form bonds at a specific length, a simple potential may be substituted to better model binding dynamics. This minimalist approach has a number of advantages, and has revealed some novel and interesting structures across the investigated parameter space.

In our coarse-grained molecular dynamics model, the semiflexible polymer is simulated as a bead-spring chain of point masses with size parametrized by $r_{\text{min}}$. Intrafilament adjacent bead-bead interactions have a strong simple harmonic potential. A bending potential between each three adjacent
beads $\Delta L$ apart parametrizes the persistence length $L_P$ or bending stiffness $K_B$ of the filament as a function of the angle $\theta$ between them,

$$\langle \cos \theta \rangle = e^{-L/L_P}, \quad K_B \approx \frac{L_P^2 k_B T}{\Delta L},$$

$$V_\theta(\theta) = K_B (\theta - 180^\circ)^2.$$ 

A modified Lennard-Jones potential $V_{LJ,r}$ with a linear regime is used to model the long-range attractive potential and minimum filament spacing of cross-linkers implicitly,

$$V_{LJ,r}(r) = \begin{cases} 
\varepsilon \left[ \left( \frac{r_{\min}}{r} \right)^{12} - 2 \left( \frac{r_{\min}}{r} \right)^6 \right], & r < r_{\min}, \\
\varepsilon \left[ \frac{r_{\min} - r}{r_{\min} - r_{\min, \text{cutoff}}} + 1 \right], & r \geq r_{\min}.
\end{cases}$$

The Lennard-Jones potential’s long range behavior is highly dependent on $r_{\min}$. The appended long-range linear regime allows for a more consistent attractive potential across different $r_{\min}$ with a cutoff distance of 440 nm limiting the range of attraction. The cutoff does not interrupt structure formation, as it is much larger than the average spacing between bundles in a network. This model allows parametrization of the binding energy (attraction) strength $\varepsilon$ and minimum coaxial distance $r_{\min}$.

Filament length $L$ is parametrized with a consistent mass/length linear density. The total mass was uniform across all simulations. Simulations were run using NAMD [30] with CUDA using a Langevin thermostat at 300 K for 200 $\mu$s with 40,000 time steps in a 3.23-$\mu$m periodic box. The system density was constant across all simulations. Visualizations were made with VMD [31] and TACHYON [32].

### B. Selection of $\varepsilon$

A single $\varepsilon$ value was chosen for an array of simulations spanning $L_P, r_{\min}$, and $L$. In order to select a value which would present a rich phase space, an array of simulations across varied $\varepsilon$, $r_{\min}$, and $L_P$ was first analyzed. At low $\varepsilon$, systems evolve too slowly, hindering network formation. At high $\varepsilon$, dynamics require a shorter integrator time step, and the sensitivity to $L_P$ is decreased. The most complex and well behaved phase space was found to occur at $\varepsilon = -0.061$ cal/mol.

### C. Structural characterization

The structure of a completed simulation may be quantitatively characterized using radial distribution function (RDF), power-law density scaling, and percolation analysis. Using a combination of these techniques, several structurally distinct phases have been identified: branching bundled network, nonbranching network, isolated bundles, and a knotted network [see Figs. 1(a)–1(c)]. Additional substructure properties can also be identified by these methods, such as internal bundle structure, mesh size, and connectivity.

A series of simulations across the parameter space ($r_{\min}, K_B, \varepsilon$, and $L$) was investigated. Quantitative phase characterization was carried out using RDF analysis, in addition to visual inspection of the simulation results. Figure 1 demonstrates the clear differences between the main observed phases. The ordered bundle phase is distinctive, with peaks in the RDF at approximately 20, $20\sqrt{3}$, 40, and $20\sqrt{7}$ nm, characteristic of hexagonal packing. A dip at 181 nm represents the space between bundles in the isolated bundles structure. This dip is not present in the other two more connected phases. The nonbranching network shows significantly weaker positional correlation between filaments, as the filaments are not arranged in bundles, in comparison to the RDF for the branching network which exhibits a clear bundle peak. An animation showing these characteristic phases is available as a supplementary video [33].

### D. Radial distribution function (RDF) calculation

The RDF represents characteristics of structure as a radially symmetric probability density function. This is calculated for a single bead $k$ in a simulation as $g_k(r) = \frac{n(r)}{4\pi r^2 dr}$ and summed over every bead as $G(r) = \frac{1}{N} \sum_{k=1}^{N} g_k(r)$ for $N$ beads in volume $V$ with bin size $dr$ and $n(r)$ beads in the spherical shell of width $dr$ and radius $r$ centered at $k$. For a bead $k$, beads on the same filament as $k$ are excluded from the calculation of $g_k$. Properly scaled, RDFS converge to 1 at high $r$. 

### E. Connectivity

The connectivity of the network formed is calculated as two values: average cluster size and average connections per filament (connectivity). A cluster is defined here as a group of filaments directly or indirectly connected by “bonds”; interfilament bonds are defined by proximity ($r \leq r_{\min} + 1$ nm), as the attraction is derived implicitly. This is done by representing the network as a logic table defining connections between filaments as determined by proximity, then parsing it recursively to calculate the values of interest. Connectivity and cluster size calculations are similarly normalized by the number of simulated beads.
The network graph in Fig. 2 could represent a network of six filaments with lines between nodes representing bonds between filaments.

This graph would be reduced to the logic table in Fig. 3, represented as an upper triangular matrix. Every logic “true” represents a connection between two nodes, i.e., \( T_{3,4} = 1 \) represents the connection between nodes 3 and 4.

The \( N \times N \) logic table representing connections between filaments is then parsed \( N \) times, starting from each element on the diagonal. For each 1 along that element’s row and column, the parsing algorithm recurses. As the table is scanned, elements at which a recursion starts are set to 0 in the local instance to prevent redundant loopback. Recursions starting along the diagonal increment cluster size ratio by \( \frac{1}{N} \), while off-diagonal recursions increment connectivity ratio by \( \frac{1}{N^2} \). The connectivity and average cluster size are thus scaled together such that a system with every filament in one cluster is represented by a cluster size value of 1. A system of \( N \) nodes can have a maximum of \( \frac{1}{2}(N^2 - N) \) links, although a connectivity value of 1 is attainable with only \( N \) links, as demonstrated by the contrast between the ring and fully connected network topologies in Fig. 4. Therefore, the connectivity value can easily exceed 1 by this definition.

F. Fractal dimension

The fractal dimension (or power-law scaling factor) has a strong relationship with the RDF. This can be calculated from the RDF by fitting the integral of \( G(r) \) to a power law, \( \sum_{r=r_1}^{r_2} G(r) \rightarrow a(r)^b \), in length scale range \( r_1 \) to \( r_2 \) where \( D \) is the scaling factor and \( a \) and \( c \) are arbitrary fit constants, or, equivalently, by the slope of the integral of \( G(r) \) plotted log-log. When used to describe structures, this value represents how the structure mass scales with space. This concept can be applied to describe the change in structure across length scales, even though a fit is only consistent over a short range. Values for \( D \) can indicate geometries such as a line (\( D = 1 \)), a plane (\( D = 2 \)), and a volume (\( D = 3 \)).

\[
T = \begin{bmatrix}
1 & 1 & 0 & 0 & 1 & 0 \\
1 & 1 & 0 & 1 & 0 & 0 \\
1 & 1 & 0 & 0 & 1 & 1 \\
1 & 1 & 1 & 1 & 0 & 0 \\
1 & 1 & 0 & 0 & 1 & 1 \\
1 & 1 & 0 & 0 & 0 & 1 \\
\end{bmatrix}
\]

FIG. 3. A logic table representing the graph shown in Fig. 2.

FIG. 4. Two systems with the same cluster size, but very different connectivity. Each node again represents a single filament.

III. RESULTS

To probe the impact of varying filament stiffness and length as a function of attractive potential, different simulations were carried out. Figure 5 shows phase diagrams for four different attractive potentials with different \( r_{\text{min}} \) values. In each of these diagrams the strength of the potential \( \varepsilon \) was kept constant, but the position of the potential well shifted in order to mimic cross-linkers of different lengths. We wanted to test the hypothesis that network structure is generally strongly dependent on filament spacing and flexibility as previously postulated for networks of actin bundles in the presence of \( \alpha \)-actinin [19].

In all cases we can see that there is a thresholdlike behavior for the formation of connected networks; both bundled and nonbundled networks only occur for filaments longer than 500 nm (also see Fig. 6). This effect is independent of other parameters in the system. Above the length threshold, we can see a clear phase sequence as a function of \( L_P \) (or \( K_B \)). At the stiff end of the range (high \( K_B \)) nonbranching networks occur, there is an intermediate range of flexibilities that favors the branching bundled network, then at low \( K_B \) the network becomes highly entangled, forming unique knotted structures, where filaments twist together within the bundles and produce more complex, disordered structures (Fig. 7). This series of phase diagrams also reveals how the phase sequence can be shifted by controlling \( r_{\text{min}} \), providing an extra degree of tunability to the system. As \( r_{\text{min}} \) is increased, filaments are forced to bind further apart. This increased filament spacing shifts the structural hierarchy towards the more complex structures, having an effect similar to an increase in flexibility. It was observed that thick coaxial filament bundles (as to exclude the knotted network structure, which exhibits noncoaxial bundling) also tend to form a hexagonal packing structure as indicated by correlation peaks in Fig. 1.

Figure 8 shows a power-law mass-density scaling analysis of the three different phases: bundled network, isolated bundles, and nonbundled network. In Fig. 8, graphs (a)–(c) show mass-density scaling for each structure (magenta, top) in comparison to slopes of fixed values (blue, bottom). For comparison, a system of rigid rods would yield a slope of 1 over a range where rod width \( < r < \) rod length. A solid material of uniform density will give a slope of 3. The flexible Gaussian chain modeled in Fig. 8(d) produces the expected fractal-like structure with a slope of 2 over two decades of \( r \), validating the calculation method.

Results show that the structures in Fig. 8 are uniform in density over longer length scales, tending towards a slope of 3 at 285, 230, and 229 nm respectively. Below this point we observe crossovers to different scaling behaviors. Short
filaments [Fig. 8(b)] demonstrate typical scaling behavior for rigid rods, with a slope of 1 down to a length scale corresponding to the bundle thickness. In comparison, the branched and nonbranched networks display similar structures with exponents of 1.3 and 1.4 respectively. Branching networks were observed to have a higher crossover when compared with the nonbranched networks, indicating a larger mesh size.

Length threshold

A length threshold was observed below which percolation does not occur. In the series of simulations shown in Fig. 6, this transition point is identified as 500 nm. This transition is expected to be largely dependent on the global density of the system; $L_p$, $\epsilon$, and $r_{\text{min}}$ showed negligible effect on this value.

IV. DISCUSSION

In a previous scaling analysis of experimentally imaged F-actin–$\alpha$-actinin system that gels at high crosslinker concentrations will shrink after formation [4,5]. This phenomenon was attributed to the formation of additional connections after percolation. To investigate this hypothesis we carried out a connectivity analysis on simulated networks in which cluster size and connectivity can be quantified over the simulation duration. Connectivity continues to increase after the system percolates, as filaments form more connected bundles, though the network structure does not significantly change [see Fig. 9(b)]. As can be seen in Fig. 9(b), we find that connectivity continues to increase monotonically after the clusters reach a maximum size (where 1 represents a cluster spanning the simulation box). Isolated bundles have a low connectivity and cluster size because they do not form a network.

FIG. 5. (Color online) Phase diagrams for an array of simulations. Filament length $L$, persistence length $L_p$, and minimum coaxial distance $r_{\text{min}}$ are varied for this set. Diagrams show phases for $r_{\text{min}}$ of (a) 10 nm, (b) 20 nm, (c) 30 nm, and (d) 60 nm. Colors and symbols indicate the different phases, with a double symbol representing a mixed state.

FIG. 6. A comparison of average cluster size across different filament lengths for $L_p = 3.23 \mu m$, $r_{\text{min}} = 20 \text{ nm}$. Network formation begins at 500 nm for this system.

FIG. 7. (Color online) A knotted network with $L_p = 3.34 \mu m$, $L = 1 \mu m$, $r_{\text{min}} = 60 \text{ nm}$ in a “licorice” and “line” view.
These results are consistent with experimental observations of contraction and gel “skin” formation [4,5] in networks of F-actin bundles and suggest slow bundle thickening as free filaments connect with the network structure. We note that the effect is observed for both branched and nonbranched networks in this system.

The dynamics of formation for the network of bundles show two distinct differences from the single filament network. The system percolates much more rapidly, and also makes more connections, plateauing at a higher connectivity [see Fig. 9(b)]. This result can be easily explained by considering the structures shown in Fig. 1. The single filament network does not form bundles and is constrained by its lack of filament flexibility once connections are formed. This limits the number of possible rearrangements at later times. The maximum connectivity reached is strongly dependent on filament flexibility.

This technique additionally highlights the minimum length required for network formation, as percolation only occurs above 500 nm (see Fig. 6). The length threshold shows a dramatic transition to a percolated system, though length has a minor role past this threshold.

This is much unlike the results presented by Chelakkot et al., who observed nearly full independence of structure from filament length in Monte Carlo simulations of rigid rods with cross-linkers [34].

A. Structural chirality

Chiral structure is innate to biopolymers, where superhelicing (helical structure from many chiral filaments) has been predicted [35,36] and observed. Bundle helices (as shown in Fig. 9(a)) were observed for 400-nm filaments with small \( L_P \) and 20–30 nm \( r_{\text{min}} \). This packing-induced chirality suggests that ex silico filament superhelicing is not necessarily a result of the geometric frustration caused by cross-linker–filament binding with helical polymer filaments. This effect may be induced with neither helical filament geometry nor discrete cross-linkers, but by simple energy minimization. Similar structural chirality has also previously been observed to emerge in simulations of discotic particles [37] and explored theoretically [35,38].

FIG. 8. (Color online) Mass-density scaling with power-law fits at various length scales. Plots (a)–(c) respectively represent the structures shown in Figs. 1(a)–1(c). Plot (d) represents a freely jointed Gaussian chain model. Inset images show only a small section of the full structure for illustrative purposes. Filaments are represented in snapshots by the links between beads, showing the filaments’ contours.

FIG. 9. (Color online) Simulations from Fig. 1 characterized by percolation analysis (b) as a time series. Solid lines represent connectivity; dashed lines represent cluster size. Data are shown for simulation parameters: blue (triangle) \( (L_P = 3.34 \, \mu m, L = 1 \, \mu m, r_{\text{min}} = 20 \, nm) \), red (circle) \( (L_P = 33.4 \, \mu m, L = 1 \, \mu m, r_{\text{min}} = 20 \, nm) \), and green (square) \( (L_P = 3.34 \, \mu m, L = 200 \, nm, r_{\text{min}} = 20 \, nm) \).

FIG. 10. (Color online) Bundle superhelicing was observed as in (a) for 400-nm filaments with \( r_{\text{min}} = 30 \, nm \). Transverse bundling was observed as in (b) with 200–400-nm filaments with \( r_{\text{min}} = 100 \, nm \).
B. Knotted network structure

The knotted network structure shown in Fig. 7 has the unique characteristic of having highly disordered binding within the network nodes. Similarly, tendrils frequently have a braided structure by which inner and outer filaments exchange places in a disordered way. This structure is observed in low $L_P$ systems with long filaments and high $r_{\text{min}}$.

C. Transverse bundle network

A novel transverse bundle network phase [as shown in Fig. 10(b)] was observed when filament length $L$ approached coaxial spacing $r_{\text{min}}$ for short filaments. This phase exhibits tendrils between network nodes in which the filaments face orthogonal to the direction of the bundle.

In summary, the phase diagrams reveal a hierarchy of complexity, isolated filaments (low complexity) ⇔ nonbranching network ⇔ branching network ⇔ knotted network (high complexity), dependent on $L$ and $L_P$. The accessible phases are tuned by varying $r_{\text{min}}$, therefore network complexity increases with both flexibility and $r_{\text{min}}$. At high flexibilities, if the filament spacing approaches the persistence length, filaments are able to weave around each other to form a fascinating knotted network structure. Shorter filaments are limited by the range of space they can sample and do not form networks. These rules form a guide for structurally tuning the fundamental properties of engineered synthetic semiflexible filament polymers.

ACKNOWLEDGMENTS

The authors would like to thank L. T. Nguyen for his significant contributions to the coarse grain F-actin model and simulation framework. We also gratefully acknowledge financial support from NSF CAREER Grants No. DMR-0852791 and No. DMR-0843934.