Topological defects produce kinks in biopolymer filament bundles

Valentin M. Slepukhin\textsuperscript{a,1}, Maximilian J. Grill\textsuperscript{b}, Qingda Hu\textsuperscript{c,\textdagger}, Elliott L. Botvinick\textsuperscript{c,d,e}, Wolfgang A. Wall\textsuperscript{b}, and Alex J. Levine\textsuperscript{e,\textdagger}\textsuperscript{g}

\textsuperscript{a}Department of Physics and Astronomy, University of California, Los Angeles, CA 90095-1596; \textsuperscript{b}Department of Biomedical Engineering, University of California, Irvine, CA 92697-2730; \textsuperscript{c}Institute for Computational Mechanics, Technical University of Munich, 80333 Munich, Germany; \textsuperscript{d}Department of Biomedical Engineering, University of California, Irvine, CA 92697-2730; \textsuperscript{e}Beckman Laser Institute, University of California, Irvine, CA 92697-2730; \textsuperscript{f}Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1596; and \textsuperscript{g}Department of Biophysics, University of California, Los Angeles, CA 90095-1596

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Bundles of stiff filaments are ubiquitous in the living world, found both in the cytoskeleton and in the extracellular medium. These bundles are typically held together by smaller cross-linking molecules. We demonstrate, analytically, numerically, and experimentally, that such bundles can be kinked, that is, have localized regions of high curvature that are long-lived metastable states. We propose three possible mechanisms of kink stabilization: a difference in trapped length of the filament segments between two cross-links, a dislocation where the endpoint of a filament occurs within the bundle, and the braiding of the filaments in the bundle. At a high concentration of cross-links, these last two effects lead to the topologically protected kinked states. Finally, we explore, numerically and analytically, the transition of the metastable kinked state to the stable straight bundle.

Significance

A common structural motif for stress-bearing intracellular structures and tissues is a network of filament bundles. When observed in optical microscopy, these bundles often show localized bends—kinks—in their contour even though the stress-free state of their constituent filaments is straight. Using a combination of analytical mechanics and large-scale, finite-element Brownian dynamics simulations, we show that the optically observable kinks are related to topological defects in the interior, nanoscale structure of the bundle. Moreover, the kinks are more compliant to bending than the rest of the bundle. As a result, defected bundle networks must contain a random distribution of soft hinges, which, being the most compliant elastic elements, control the low-energy excitations of these bundle networks.

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1To whom correspondence may be addressed. Email: valentinslepukhin@physics.ucla.edu.

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number and position of these quenched defects, which act like soft hinges in a 3D network of bundles that behave more like stiff beams.

Topological defects are well known in condensed matter, including, for example, disclinations in nematic liquid crystals and dislocations in crystalline solids (3–5). Defect motion plays a dominant role in the plastic deformation of many solids. Dislocations and disclinations are topological defects; their removal requires a system-sized reorganization of interatomic bonds. The defects in filament bundles share this feature. They cannot be removed without breaking a number of cross-links proportional to the bundle length (we consider the filaments to always be unbreakable). This feature ensures that the defects are long lived on the scale of the thermal undulations of the bundles themselves.

In our observations of collagen networks, we find kinked bundles, whose contour we quantify by measuring their local curvature using light microscopy. Due to their connection to the network, we cannot be certain that these kinks are not in some way related to elastic stress in the network. To address this question, we used large-scale Brownian dynamics simulations to study kinking in quenched filaments with force- and torque-free boundary conditions, finding that quenched defects produce a statistical distribution of kinks similar to those observed in the experiment. Using the simulations, we are also able to measure the reduction of the bundle’s local bending modulus at the location of the defects and observe the motion of the defects along the bundle. Finally, we present theoretical calculations using a simple model of semiflexible filaments that demonstrate the relationship between defects and kinks in the bundle. Moreover, we analytically determine (and test via simulation) the time evolution of the number of defects in a bundle as they slowly anneal through defect–defect annihilation or by diffusion off the ends.

We first report our observations from light microscopy of kinks in collagen bundles and compare these kinks with those from numerical simulations. We then present a general discussion of the three types of defects and demonstrate that the minimum energy state of the defected bundle can be kinked. We explore defect dynamics, estimating the lifetime of a kink and the number of kinks in a bundle as a function of time, which we compare to simulation. To properly describe interaction of braiding type defects, we use the theory of the braid group; some relevant background is provided in SI Appendix, section 3.

Results

Experiment. The nanoscale structure of collagen is quite complex (6, 7). Small fibrils bind together to form larger fibrils, which, in turn, bind together to form fibers, which we observe in light microscopy. Given that these fibers associate rapidly and strongly with local bonds, collagen fibers are a good place to look for quenched defects in bundles and kinks, if such sharp bends of the bundle indeed result from those defects (8). In fact, kinked collagen bundles have been observed previously (9–11) using electron microscopy. These observations leave the possibility that the kinks observed in a single snapshot of a dynamic, flexible structure may be consistent with thermal undulations about a straight equilibrium state, rather than long-lived sharp bends (12). To address this question, we made multiple observations of collagen bundles in an aqueous environment to determine whether the time-averaged state of the bundles includes kinks.

We reconstituted pepsin-extracted type I bovine collagen and fluorescently labeled and imaged individual bundles. In Fig. 1,4, we show 50 superimposed images of a single bundle (white on a black background) taken 0.5 s apart and showing three persistent kinks, which confirms that they are indeed long-lived structures. Green lines indicate the measurement of a kink angle. We measure the 2D projection onto the microscope’s focal plane of the physical kink angle in three dimensions. We accept kink observations only when at least about 3 microns of bundle is observable on either side of the kink. In order for the image of the bundle to extend away from the kink on both sides, our reported kinks must lie in a plane making, at most, a small angle with respect to the focal plane. As a result, the discrepancy between our observed kink angle distribution and the physical one is quite small. We find less than 10% discrepancies between the projected and 3D, physical angle distribution when measurements of collagen bundles using fluorescence microscopy (A–C) and simulations (D–F). (A) Fluorescence image of a collagen bundle with noticeable kinks. Green traces show a measurement of the kink angle, given as angular deviation from straight. (B) Mean curvature (black) of a collagen bundle over 50 images. Red lines show the spread of curvature in time (1 SD). Blue arrows mark the locations of high curvature. (C) Histogram of mean kink angles for all measured bundles (n = 74). (D) A 2D simulation of bundles with reversible cross-linkers showing the bundle centerline (black dashed line), individual filaments (blue and red), and braids (black dots). (E) Mean of the curvature over 100 configurations (black). Red lines indicate 1 SD. (F) Kink angles from simulation for two-filament bundles in two dimensions and seven-filament bundles in three dimensions measured in the same way as in C.

![Fig. 1. Measurements of collagen bundles using fluorescence microscopy (A–C) and simulations (D–F). (A) Fluorescence image of a collagen bundle with noticeable kinks. Green traces show a measurement of the kink angle, given as angular deviation from straight. (B) Mean curvature (black) of a collagen bundle over 50 images. Red lines show the spread of curvature in time (1 SD). Blue arrows mark the locations of high curvature. (C) Histogram of mean kink angles for all measured bundles (n = 74). (D) A 2D simulation of bundles with reversible cross-linkers showing the bundle centerline (black dashed line), individual filaments (blue and red), and braids (black dots). (E) Mean of the curvature over 100 configurations (black). Red lines indicate 1 SD. (F) Kink angles from simulation for two-filament bundles in two dimensions and seven-filament bundles in three dimensions measured in the same way as in C.](https://doi.org/10.1073/pnas.2024362118)
testing this procedure with simulated data—see SI Appendix, section 1.

Seventy-four kink angle measurements from 43 bundles are summarized in Fig. 1C. The trace of the local curvature versus arc length along the bundle shown in Fig. 1B quantifies the points of high persistent curvature as indicated by the blue arrows. These local curvatures were computed by discretizing the contour using the intensity pixels in each image and computing the curvature from a cubic spline fit to these data. More details are given in SI Appendix, section 1. Repeating this procedure for other bundles, we observed kinks and determined their mean kink angles by averaging again over up to 50 repeated measurements of each kink angle. They showed temporal fluctuations with a nonzero mean. We present the distribution of kink angles for 74 kinks in Fig. 1C. There were larger variations between kink angles measured across multiple bundles than in the thermal fluctuations of a given kinked bundle. The distribution of these time-averaged kink angles has a mean at 26° and a spread of ±11°. There were larger variations between kink angles measured in different bundles, which are all related to a mismatch between amount of filament and length taken up per unit length of the bundle. These are 1) braids, that is, rearrangements of filaments within the bundle, and 2) loops where one filament stores excess length by looping out of the bundle and then reattaching to it. Both braids (actually pseudo-braids, as described below) and loops are shown in Fig. 2B from a 2D simulation of two filaments where the filaments are allowed to cross each other but cannot untwist. This special setup is motivated by the fact that it is the simplest system capable of supporting a loop or a pseudo-braid. The pseudo-braid is a projection of a braid onto two dimensions and is the mechanical analog of a true braid in three dimensions. As will be shown in Kinking Theory, the energetics of the two-filament pseudo-braid is equivalent to that of a true 3D braid of three filaments when the two filaments making up the pseudo-braid have different bending moduli. The simplest system that supports true braiding defects is a three-filament bundle in three dimensions, shown in Fig. 2C. Inset A shows the typical structure of a loop in a larger bundle. There is also a third type of defect, 3) a dislocation in which a filament end appears within the bundle. This defect was not created in our simulations, due to the fact that we started the system with equal length filaments whose ends were initially aligned at one end of the simulation box. In the simulations, we concentrate on braids and loops. Defects observed in the 3D simulations were found to trap torsional as well as bending energy. The torsional torques measured in simulation were smaller than the bending torques that lead to kinking. We revisit this point in the discussion of our analytical model in The model. In the following sections, we first analyze the curvature of the bundle centerline as well as the kink angles resulting from braids and loop defects, and then investigate the dynamics of the defects, that is, how they move along the bundle and potentially interact with each other.

Curvature and kink angles of defected bundles. Fig. 1D shows a typical configuration of the minimal bundle setup with two filaments (blue and red) in two dimensions. The bundle centerline (black dashed line) is computed as the average of the two filament centerlines, and braids (black dots) are detected by the
crossings of the filament centerlines. A movie of the bundle’s dynamics can be found in Movie S2. The curvature of the bundle’s centerline as a function of centerline arc length is plotted in Fig. 1E, showing both the mean (black) and the standard deviation range (red lines) of the curvature computed from 100 simulation snapshots with a time interval of 1 s. Close to the midpoint of the bundle in the range of arc lengths $10 \mu m < s < 12 \mu m$, we observe two peaks in the curvature that are clearly visible as a double kink in the bundle configuration shown in Fig. 1D. These can be explained by the braid and loop defects there. The standard deviation of the curvature is increased by about one order of magnitude in this defected region, indicating a local increase in angular fluctuations at this point. This is a direct measure for the decreased effective bending modulus of the bundle in these defected, non–cross-linked regions. Using the relation between the thermal fluctuations of the local curvature and the bending modulus, we estimate a decrease in the effective bending modulus of about two orders of magnitude. Apart from the bending modulus, we estimate a decrease in the effective relation between the thermal fluctuations of the local curvature and the bundle in these defected, non–cross-linked regions. Using the relation between the thermal fluctuations of the local curvature and the bending modulus, we estimate a decrease in the effective bending modulus of about two orders of magnitude. Apart from the locally decreased bending modulus, such a defect most likely also leads to an anisotropy in the bundle’s bending mechanics, which breaks another basic assumption of the ideal bundle as a single, thick filament. Similar features in the curvature data are observable for the second braid of this bundle at approximately $s = 6 \mu m$ of this bundle. More examples are found in the other simulation runs. Additional results showing the curvature along the bundle at different time points are provided in SI Appendix, Fig. S5.

The histogram of measured kink angles over a total of 12 simulations is shown in Fig. 1F. Here, we applied the same procedure for the angle measurements as described for the experimentally obtained microscopy images in SI Appendix, section 1. The 3D simulation results were rotated such that the bundle centerline tangents left and right of the kink lie approximately within the image plane. The distribution of 72 kink angles for the two-filament bundle has a mean of $27^\circ$, with a standard deviation of $14^\circ$ and values ranging from $4^\circ$ to $77^\circ$. The kink angle distribution for larger bundles with seven filaments in three dimensions demonstrates a trend toward smaller angles and a more narrow distribution with $20\pm 8^\circ$ (mean ± standard deviation). Big bundles with up to 225 filaments will be investigated in more detail below.

**Dynamics and interactions of defects.** We now use our simulations to study dynamics on longer time scales, where we expect to see the motion of defects along the bundle and their annealing as the metastable, defected bundle slowly relaxes. To facilitate these observations, we need to speed up the motion of the defects by doubling the linker unbinding rate in our simulations to $k_{\text{off}} = 6 \text{ s}^{-1}$. At this rate, the motion of defects is still much slower than the undulatory fluctuations of the bundle, but now defect motion is moved into a time scale accessible by simulation, which covers $1,000 \text{ s}$. Fig. 3 shows an example of how the (defected) configuration of a two-filament bundle evolves over time. We plot the position (measured by arc length) of braids (red dots) and cross-links (blue dots) along the bundle horizontally, with time increasing vertically. The resulting red tracks record the world lines of the braids over a simulated period of 500 s. The white vertical scars show cross-linker gaps in the otherwise densely cross-linked bundle. Due to a small offset between the filaments, there is a nearly persistent gap in cross-linking at the left end of the bundle where one filament stops. Cross-linkers appear in this gap because one filament slid far enough past the other to wrap around and briefly cross-link to the other one due to the periodic boundary conditions of the simulation box. We observe a pair of braids located near the bundle midpoint first emerge after $\sim 20 \text{ s}$ during the initial quench of the bundle. This time scale for the formation of the quenched bundle is typical and consistent with observation of the initial growth of the number of double bound cross-linkers; that number rapidly increases from zero at the beginning of a simulation and plateaus around 20 s, indicating the maturation of the defected bundle—see SI Appendix, Fig. S4 for further details on bundle self-assembly. Once the bundle has formed, the two braids in the middle approach each other and appear to annihilate, leaving a low cross-linker density region within the bundle during the time period of $100 \text{ s} < t < 270 \text{ s}$. After that time, a new pair of braid defects form. These slowly separate as more and more cross-links are formed between them.

The single braid close to the bundle’s right end diffuses until it approaches the far right end of the bundle at $s \approx 19 \mu m$ and at time $t \approx 280 \text{ s}$. Here it vanishes by diffusing off the open end. The filaments simply uncross, and new cross-links are
established between the unbraided filaments. Looking more carefully, one may observe a similar phenomenon on the left edge of the bundle. Immediately after the quench, there are actually four braids on the bundle, as indicated by the picture of the system at time slice $t = 34\ \text{s}$. Almost immediately after this time and long before the next bundle configuration image at $t = 200\ \text{s}$, that leftmost braid diffuses off the left end of the bundle. The final state of the bundle at $t = 500\ \text{s}$ shows a bent configuration where the localized bend near the center of the bundle is due to the two interacting braids that remain in the system. For these parameter values, the typical lifetime of a defect is hundreds of seconds.

**Big bundles.** Motivated by the fact that the number of filaments in a biopolymer bundle is likely to vary from $\mathcal{O}(1)$ to $\mathcal{O}(10^3)$, we explored simulations of very big bundles in three dimensions. Fig. 4A shows a self-assembled bundle with 225 filaments (white) and approximately 16,000 cross-links (pink). To rule out the influence of the initial arrangement of filaments in plane perpendicular to the bundle’s mean tangent, we ran simulations with filament end points placed on a square grid in addition to the hexagonal grid. We observed no significant differences.

The large bundle’s structure is hierarchical; one can identify more-tightly bound subbundles that form loops and braids with each other along the bundle’s length. As observed already in 25-filament bundles, its centerline remains rather straight, while the subbundles show the characteristic kinks observed in the smaller bundles. One possible explanation for the rather straight form of the big bundles is their smaller aspect ratio as compared to the small bundles; in other words, very large bundles may well show kinks over longer distances, since such kinks require higher energy and are thus statistically less probable or more sparsely distributed defects. Simulations of big bundles with the same aspect ratio as the smaller ones remain computationally prohibitive. We observe, in the large bundle, a large hole created by a subbundle loop defect. Its appearance is strikingly similar to our experimental images of collagen bundles in Fig. 4B. Those parts of the images showing the hole defect are magnified and compared side by side in Fig. 4, Insets.

![Fig. 4. Hole defect observed in simulation and experiment. (A) A 3D simulation of a big bundle with 225 filaments (white) and approximately 16,000 cross-links (pink). (B) Fluorescent confocal laser scanning image of a collagen bundle. Those parts of both images showing the hole defect are magnified and compared side by side in Insets.](https://doi.org/10.1073/pnas.2024362118)

**Kinking Theory.**

**The model.** We now examine the energetics of kink formation using a simple model consisting of a bundle of $n$ inextensible, semiflexible filaments connected by cross-links. The filaments’ elasticity is controlled by a bending modulus $\kappa$. The filaments’ structure is an assembly of a single bending modulus $\kappa$. The filaments are arranged so that their mean tangent directions are parallel along the $z$ axis. In a cross-section normal to this direction (the $yz$ plane), the filaments’ centers lie on a triangular lattice with a lattice constant equal to the size of the cross-linkers. The cross-linkers are assumed to locally constrain both the distance between the cross-linked filaments and their crossing angle, so that the cross-links are normal to the filaments to which they bind. We further assume that the cross-linking is reversible; that is, they bind and unbind from the filament bundle so that the cross-linker density within the bundle remains in chemical equilibrium with a solution of free cross-linkers at a fixed chemical potential. Previous work has shown that thermal undulations of the filaments induce Casimir forces between cross-links (19, 20) and cause the transition between states of free filaments and a densely cross-linked bundle to be a discontinuous or first-order phase transition rather than a smooth crossover. Here we work at chemical potentials above this transition so that we may assume dense cross-linker coverage; hereafter, we neglect Casimir interactions and other fluctuation-induced effects.

If all the bundle’s filaments have the same length, the energetic ground state is a straight bundle with as many cross-links as possible. However, if at least one of the filament’s length differs from those of others, the straight bundle configuration will necessarily have a defect where a filament’s end occurs within the bundle. That dislocation defect may, in fact, be unstable toward forming a kink in the bundle’s interior, leading to a kink in the elastic ground state of the system (we explore this point in Dislocations).

When we consider metastable states, there are many more options. If removing a defect in the structure of cross-linked straight filaments requires uncoupling a large number of cross-links, the lifetime of that defect may exceed the time of the experiment. We divide such defects into two groups: defects due to the deviation of the filament from its straight state (loop) and the effects due to the permutations of the filaments (braiding). We study the simplest cases of these effects in Loops and Braids, respectively.

In all these cases, the energy of the bundle can be written as the sum of two terms: the bending energies of the $n$ constituent filaments and the energy of their chemical interactions with the cross-links,

$$E = \sum_{i=1}^{n} \int ds \left\{ \frac{\kappa_i}{2} \left( \partial_t \hat{t}_i \right)^2 + \mu \right\},$$

where $\hat{t}_i$ is unit tangent vector of filament $i$. The integral is taken over the piece of the filament without cross-links, which generates the term $\mu$ equal to the work of unbinding the cross-links in this piece, where $\mu$ is a linker binding energy per unit length. Eq. 1 implicitly assumes a linear elastic response of the material to bending deformation in that the bending torque is proportional to the bending angle. The parameterization of local curvature, however, is exact, even for large bending. In essence, we use the usual assumption (21) that, due to the thinness of the filaments, there are no large strains within the filament cross-section even at large curvatures, so constitutive bending nonlinearities may be neglected even for highly deformed filaments.

Since we assume that the cross-links completely fix both angle and positions of the filaments, the piece of the filament with cross-links is straight and parallel to the whole bundle. We now minimize the bundle’s energy subject to boundary conditions that enforce the presence of one or more defects. If a kinked configuration minimizes this energy, we conclude that elasticity...
theory predicts a kink. This calculation will also determine the optimal size (length) and bending angle of the kink, which we report below. All such calculations assume that the defects do not trap filament torsion. We note, from simulation, that typical defects include some torsional deformations. As a result, our calculations represent the minimum energy configurations of each defect. We anticipate there to be a continuous spectrum of excited defect states associated with increasing torsional energy. We now perform this minimization for the three different types of defects.

**Loops.** We start with the simplest case of a two-filament bundle, forming a loop defect by demanding that the filaments have disparate lengths $L_1 \neq L_2$ between consecutive cross-links. What results is the bending of the whole bundle to form a kink (Fig. 2B). This approach generalizes to $n$-filament bundles, and can be adapted to large bundles in which two subbundles form a loop. To simplify this calculation, we take the size of the cross-links and the filaments’ diameter (whose sum is $a$) to be zero. In case of loops, the excess trapped length in the defect is not principally controlled by that length, so, in this case, the $a = 0$ limit both is reasonable and simplifies the calculation. Then the boundary conditions for the positions of the ends of the filament are integral conditions on the tangent vector,

$$\int_{-L_1 / 2}^{L_1 / 2} ds \hat{t}_1 (s) = \int_{-L_2 / 2}^{L_2 / 2} ds \hat{t}_2 (s),$$

and boundary conditions for the tangent vector determine the kink angle $\phi$, which is the total bend of the tangent across the structure. We pick a reference frame so that these boundary conditions are symmetric,

$$\hat{t}_2 (L_1) = \hat{t}_2 (L_2) = \left( \frac{\cos (\phi / 2)}{\sin (\phi / 2)} \right).$$

Minimizing the energy from Eq. 1 in the limit of small filament bending ($L_b \ll 1$), we obtain a lengthy self-consistent equation for the angle $\phi$ (see SI Appendix, section 3), which can be simplified in the case of the equal bending moduli $\kappa_1 = \kappa_2 = \kappa$ to

$$\phi = \gamma \left( \frac{\Delta L}{\mu_2} \right)^{1 / 3},$$

with the numerical constant $\gamma \approx 0.93$, and $\Delta L = L_2 - L_1 \neq 0$. We verified these results by minimizing the energy numerically (Fig. 6A). Loops produce a continuous spectrum of kink angles that grow as the cube root of their length mismatch. As expected, an increase in the bending modulus suppresses this kink angle, while an increase in the linker binding energy increases it by shrinking the extent of the gap in the cross-linking. We now turn to braids.

**Braids.** The simplest model of braiding in three dimensions requires three filaments. Braiding of two filaments in three dimensions can be undone by twisting the bundle about its long axis; it is not topologically protected (the relationship between braiding and rotation is discussed in more detail in SI Appendix, section 3). The minimum energy configuration of three cross-linked filaments with the same length will be a right prism with an equilateral triangle as its base. We choose a coordinate system so that the $x$ axis lies parallel to the filaments, filaments 2 and 3 are in the $xz$ plane, and filament 1 is above that plane. To introduce a braid, we require filament 1 to go from above to below the $xz$ plane (Fig. 2C). This configuration is metastable, since we need to decouple all the cross-links on one side to get to the minimal energy configuration. There is no rotation of an end of the bundle that will eliminate the braid.

Since the cross-links fix both relative angle and position of the filaments, filament 1 cannot be connected to filaments 2 and 3 by the cross-links in the defect core; however, filaments 2 and 3 can remain cross-linked. Thus, filaments 2 and 3 behave as one combined filament $2'$ with double the bending stiffness, and remain in the $xy$ plane like filament 1. As a result, a true three-filament braid in three dimensions is energetically equivalent to a two-filament pseudo-braid, as introduced in our simulations.

The boundary conditions on the vector $l$ are the same as in the previous case, Eq. 3, but the displacement boundary condition differs, incorporating the finite filament radius and cross-linker length $a$, which is necessary for the braid to trap excess length. We find

$$\int_{-L_1 / 2}^{L_1 / 2} ds \hat{l}_1 (s) = \int_{-L_2 / 2}^{L_2 / 2} ds \hat{l}_2 (s) + 2a \cos (\phi / 2) \hat{y}.$$  \[5\]

Unlike in looping, we do not fix the filament length mismatch $\Delta L = L_2 - L_1$, but instead allow it to vary to relax the braid’s energy. It is conceivable that one may encounter higher-energy braids in which braiding and an excess of trapped length (looping) coexist. We do not study this case here.

We observe that braids should generate local bending, at least in the limit of a sufficiently soft bending modulus. The binding free energy (chemical potential difference between free and bound linkers) acts as an effective tension on the bundle. Setting the bending modulus to zero and fixing the length of the braided region, the solution for the filament contours inside the braid will be straight lines. In this configuration, linker-induced tension generates a torque that increases the kink angle of kinked configurations (Fig. 5A). To stabilize this angle at a finite value, we must include finite bending compliance. We do so now, turning to the full calculation.

Calculating the energy of the braid as a function of the kink angle $\phi$, we find that kinking is controlled by the dimensionless parameter

$$\zeta = \frac{\mu a^2}{\kappa_2},$$

where $\mu$ is the cross-linker binding energy per unit length, $\kappa_2$ is the bending modulus of filaments of type 2, and the length $a$ is the normal distance between the centerlines of a pair of
bound filaments. This length can be interpreted to be the sum of the radii of the filaments and the length of the cross-linking molecule. $\zeta$ is the ratio of the linker binding energy per unit length $\mu$ to an energy per length set by bending on the scale of the interfilament separation, $\kappa/a^2$. In general, we expect larger $\zeta$ values to lead to kinking. Below, we verify this intuition. Larger values of the linker binding energy and interfilament separation increase filament bending in the defected region. This bending, which is opposed by $\kappa$, provides the torques necessary to produce the kinks. The nonphysical limit of zero interfilament distance ($a \to 0$) implies that braids involve no filament bending and thus generate no bending moments necessary for kinking.

We prove, in SI Appendix, section 3, that, for small values of $\zeta$, the energy is minimized at $\phi = 0$; that is, there are no kinks. For large values of $\zeta$, the minimum energy states are kinked ($\phi > 0$). We examine this transition in more detail for the case $\kappa_1 = \kappa_2$. We assume symmetric bending, $\kappa_1(s) = -\kappa_2(-s)$, noting that numerical solutions of the minimization show that the symmetric solution based on this ansatz indeed identifies the global energy minimum. We obtain (see SI Appendix, section 3)

$$\lambda^2 \sin^2(\phi/2) = \frac{8\kappa^2}{a^2} (\zeta - 2),$$

where we introduced a Lagrange multiplier $\lambda$ to enforce the $\dot{y}$ component of Eq. 5, which plays the role of the tensile force in $\dot{y}$ direction. When $\zeta$ increases to two, there is a second-order transition at which the kink angle grows continuously from zero as $\zeta$ increases. Near the critical point $\zeta = 2 + \alpha$, $\phi \approx \sqrt{\zeta}$. Numerical minimization of the energy from Eq. 1 leads to the same result (Fig. 6B). In that figure, we also see (red dots) that, when the two filaments have differing bending moduli, there is a first-order kinking transition where the kink’s angle jumps discontinuously from zero at the critical value of $\zeta$.

**Dislocations.** The simplest dislocation requires a bundle of four filaments in three dimensions where one of the four ends within the bundle. The stable state of four filaments is a right prism formed by a base of two equilateral triangles sharing one edge, as shown in Fig. 5B. We label these triangles as 1-2-4 and 2-3-5; only filaments 1 and 3 are not cross-linked to each other. If either filament 1 or 3 ends within the bundle, the configuration remains stable because the other three form a stable three-filament prism. But, if another filament ends, for example, filament 4, the remaining filaments must deform to recreate a cross-section with an equilateral triangle (Fig. 5B). Due to cross-linker constraints, the distortion associated with the defect must locally remove cross-links between two of the filaments. Without loss of generality, we demand that filaments 2 and 3 remain cross-linked. Calculating the energy associated with this defect is complicated by the fact that there is no mapping to a 2D version of the distortion. To gain immediate insight, it is helpful to consider, momentarily, the unphysical case of zero filament bending modulus. Then filaments 2 and 3 remain straight, but filament 1 makes two right-angle bends at the defect to move to the location of the missing filament 4 and thereby maximize cross-linking. If we now reintroduce a finite bending modulus, this localized dislocation will spread out along the bundle to decrease bending energy at the expense of reducing the maximal cross-linking shown in Fig. 5B. A force pair is also introduced by filament 1’s bending (shown in the figure as black arrows) which produces a torque causing the entire bundle to kink. We perform numerical minimization of the energy assuming that filaments 2 and 3, being cross-linked everywhere, form a ribbon that can bend in the direction perpendicular to its plane with bending modulus $\kappa_{\text{ribbon}} = 2\kappa$, but is absolutely rigid in the direction parallel to its plane. The results of our qualitative analysis above are consistent with the quantitative energy minimization (Fig. 6C)—the maximum of the kink angle is observed to be at a finite value of $\zeta$. Zero and infinite values of that parameter lead to a zero kink angle.

**Defect Dynamics.** Over times significantly longer than those associated with the undulations of the bundle, defects can move along the bundle and interact. These dynamics require multiple cross-linker binding/unbinding events. As a result of these events, defects move diffusively and may eventually fall off the ends of the bundle. In the case of dislocations and braids, defects may combine or annihilate. For the latter type, these interactions are controlled by the structure of the braid group.

Consider two braids—a braid/antibraid pair—separated by $N$ cross-links. Since these defects would annihilate if the intervening cross-links were removed, we may expect this pair might vanish if their separation becomes sufficiently small. The braids are motile, with a diffusion constant set by the linker detachment rate $k_{\text{det}}$, and do not strongly interact when separated by lengths greater than the defect core size. The probability density $p(n, t)$ of there being $n$ cross-links separating the two defects at $t$ then obeys a diffusion equation,

$$\frac{\partial p(n, t)}{\partial t} = 2k_{\text{det}} \frac{\partial^2 p(n, t)}{\partial n^2}.$$

Using a well-known result for the first mean passage time (22), the mean lifetime of this braid/antibraid pair is
order to compensate for that distortion. This mechanism is reminiscent of the relaxation of a flexible hexatic membrane in the vicinity of a disclination (24). There, a topological defect relaxes local strain via a puckering of the membrane that produces long-ranged Gaussian curvature. Here the distortion of the bundle may be entirely localized in a sharp bend.

In our experiments, we found that 4% of the observed collagen bundles had one or more kinks and that these kink angles had a mean of 26°, but were quite varied, ranging up to 74° in the sample of 74 kinks studied. When we consider that loop defects can produce a continuous distribution of kink angles, it seems natural to suppose that this defect is the predominant cause of kinking. The number of observed kinks is likely an underestimate of the real system, due to the limitations of our imaging that shows only those bundles lying in the imaging plane. Only kinks oriented so that the bundle bends within the imaging plane are observable.

The kinks associated with both braids and dislocations are expected to be narrowly distributed at angles set by the number of filament lengths in the bundle, since these defects produce fixed kink angles that depend only on that number, the cross-linker binding energy, and bending moduli of the filaments. For a fixed number of filaments, both dislocations and braids produce kink angles that depend on only a single dimensionless number ζ = μa2/κ. In the case of braids, the angle grows from zero at a critical value of ζ, which depends on the number of filaments but is roughly of order unity. Looking at stiff F-actin cross-linkers like α-actinin, we find that ζ ≈ 0.1; it is too small for braids to generate kinks. We do, however, expect braids to be associated with kinks in softer filament systems such as DNA condensed by polyvalent counter ions (25, 26) or cross-linked intermediate filaments (27, 28) where ζ ≈ 10 to 100. Currently, our understanding of collagen bundle cross-linking is less precise; our estimate in this system is that ζ ≈ 0.1 (see SI Appendix, section 1). This suggests that loops certainly should produce kinks and that braids should not. However, given the large uncertainty in our estimate of ζ, it is conceivable that braids are also kink-generating defects. Of course, even if braids do not produce kinks, we expect them to be present and to produce high-flexibility “hinges” in the bundle. Dislocations always generate kinks, but the kink angle is appreciable only when ζ ≈ 1. We surmise (with the same caveats regarding the uncertainty of ζ) that dislocations are unlikely to be responsible for the experimentally observed kinks in the collagen bundles.

Another argument for loop-controlled defects in collagen is a presence of z-shaped double kinks (see SI Appendix, Fig. S7 for the examples), which can be attributed to slippage between two filaments in a bundle such that they produce a pair of loops. The lengths stored in this pair are such that, after the two loops, the filaments once again have no length mismatch.

The lifetime of these defects appears to be significantly larger than the characteristic time of thermal undulations of the filaments and longer than the typical observation time in experiments. This is supported by the experimental data, where kink annihilation or diffusion to the ends is never observed. When we study kink dynamics via simulation on the time scales significantly longer than those covered by experiment, we observe their diffusion, sticking, and annihilation, which one expects from the thermal theory. Specifically for braids, we find that their motion is consistent with particles diffusing in one dimension, with interactions obeying the rules of the braid group. We speculate that bundles under compression may relieve stress by the pair production of braid/antibraid pairs in a manner resembling the Schwinger effect (29, 30).

Examining Fig. 4 leads us to speculate that very large bundles of many filaments might be considered to be smaller bundles composed of more weakly bound subbundles, which are themselves composed of the original filaments. If we may consider
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1. M. Bathe, C. Heussinger, M. Claessens, A. Bausch, E. Frey. Cytoskeletal bundle bending, buckling, and stretching behavior. arXiv Preprint (2020). https://arxiv.org/abs/bio/1007.0402v3 (Accessed 13 March 2021).

2. C. Heussinger, M. Bathe, E. Frey. Statistical mechanics of semiflexible bundles of wormlike polymer chains. Phys. Rev. Lett. 99, 048101 (Jul 2007).

3. P. M. Chaikin, T. C. Lubensky. Principles of Condensed Matter Physics (Cambridge University Press, 1995).

4. M. Kleman, The Topological Classification of Defects (Springer US, Boston, MA, 1995).

5. D. R. Nelson, Defects and Geometry in Condensed Matter Physics (Cambridge University Press, 2002).

6. M. D. Shoulders, R. T. Raines. Collagen structure and stability. Annu. Rev. Biochem. 78, 929–958 (2009).

7. M. J. Buehler. Nature designs tough collagen: Explaining the nanostructure of native collagen fibrils. Proc. Natl. Acad. Sci. U.S.A. 103, 12285–12290 (2006).

8. P. Kannus, Structure of the tendon connective tissue. Scand. J. Med. Sci. Sports 10, 312–320 (2000).

9. M. Franchi, V. Ottani, R. Stagni, A. Ruggeri. Tendon and ligament fibrillar crimps give rise to left-handed helices of collagen fibrils in both planar and helical crimps. J. Anat. 216, 301–309 (2010).

10. S. P. Reese, N. Farhanger, R. Poulsen, G. Parkman, J. A. Weiss. Nanoscale imaging of collagen gel with focused ion beam milling and scanning electron microscopy. Biophys. J. 111, 1797–1804 (2016).

11. T. Gutmans et al., Evidence that collagen fibrils in tendons are inhomogeneously structured in a tubelike manner. Biophys. J. 84, 2593–2598 (2003).
24. J.-M. Park, T.C. Lubensky, Topological defects on fluctuating surfaces: General properties and the Kosterlitz-Thouless transition. Phys. Rev. 53, 2648–2664 (1996).
25. G.S. Manning, The persistence length of DNA is reached from the persistence length of its null isomer through an internal electrostatic stretching force. Biophys. J. 91, 3607–3616 (2006).
26. E. Raspaud, M. Olvera de la Cruz, J.-L. Sikorav, F. Livolant, Precipitation of DNA by polyamines: A polyelectrolyte behavior. Biophys. J. 74, 381–393 (1998).
27. E. E. Charrier, P. A. Janmey, Mechanical properties of intermediate filament proteins. Methods Enzymol. 568, 35–57 (2016).
28. E. Haimov, R. Windoffer, R. E. Leube, M. Urbakh, M. M. Kozlov, Model for bundling of keratin intermediate filaments. Biophys. J. 119, 65–74 (2020).
29. J. Schwinger, On gauge invariance and vacuum polarization. Phys. Rev. 82, 664 (1951).
30. J. Schwinger, The theory of quantized fields. 5. Phys. Rev. 93, 615–628 (1954).
31. G.M. Grason, R. Bruinsma, Chirality and equilibrium biopolymer bundles. Phys. Rev. Lett. 99, 098101 (2007).
32. G.M. Grason, Braided bundles and compact coils: The structure and thermodynamics of hexagonally packed chiral filament assemblies. Phys. Rev. E 79, 041919 (2009).
33. G.M. Grason, Colloquium: Geometry and optimal packing of twisted columns and filaments. Rev. Mod. Phys. 87, 401–419 (2015).
34. C.P. Broedersz et al., Cross-link-governed dynamics of biopolymer networks. Phys. Rev. Lett. 105, 238101 (2010).
35. K.W. Müller et al., Rheology of semiflexible bundle networks with transient linkers. Phys. Rev. Lett. 113, 048301 (2014).
36. A.D. Doyle, Generation of 3D collagen gels with controlled diverse architectures. Curr. Protoc. Cell Biol. 72, 10.20.1–10.20.16 (2016).
37. E. Reissner, On finite deformations of space-curved beams. Z. Angew. Math. Phys. 32, 734–744 (1981).
38. J.C. Simo, A finite strain beam formulation. The three-dimensional dynamic problem. Part I. Comput. Methods Appl. Mech. Eng. 49, 55–70 (1985).
39. M. Maier et al., A single charge in the actin binding domain of fascin can independently control the linear and non-linear response of an actin bundle network. Euro. Phys. J. E 38, 50 (2015).
40. BACI: A comprehensive multi-physics simulation framework. https://baci.pages.gitlab.lrz.de/website. Accessed 24 November 2020.
41. K. W. Müller et al., Rheology of semiflexible bundle networks with transient linkers. Phys. Rev. Lett. 113, 048301 (2014).