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The results agree with ultrastructural findings and rationalize for the commonly observed looped intermediate filament shapes.

PACS numbers: 87.16.aj, 82.35.Pq, 87.15.-v

The Helical Superstructure of Intermediate Filaments

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Intermediate filaments are the least explored among the large cytoskeletal elements. We show here that they display conformational anomalies in narrow microfluidic channels. Their unusual behavior can be understood as the consequence of a previously undetected, large scale helically curved superstructure. Confinement in a channel orders the otherwise soft, strongly fluctuating helical filaments and enhances their structural correlations, giving rise to experimentally detectable, strongly oscillating tangent correlation functions. We propose an explanation for the detected intrinsic curving phenomenon - an elastic shape instability that we call autocoiling. The mechanism involves self-induced filament buckling via a surface stress located at the outside of the cross-section.

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The integrity and dynamics of biological cells delicately depend on the mechanical response of their cytoskeletal ton consisting of actin filaments, microtubules and intermediate filaments (IFs) \cite{1,2}. While they have been experimentally probed in many different ways, a full understanding of their properties still remains a challenge. This is particularly true for the IFs - the least studied of. Experiments probing their elastic response have shown that they are the most flexible and extensible of the filaments of the cytoskeleton \cite{3,5}. Bio-filaments are usually modeled as semiflexible polymers characterized by their persistence length. For vimentin, a prominent member of the IF family, this model was used in combination with additional information about the filament’s microstructure. For instance, we observe the transient formation of rings and oscillatory shapes (see Fig. 1). However, the most prominent manifestation of this anomaly manifests itself in a strongly oscillating tangent correlation function for individual filaments, in sharp contrast to the behavior characteristic for a semiflexible chain under lateral confinement \cite{11}. In this paper we will show that the data can be completely rationalized by assuming that IFs behave like squeezed helical filaments under lateral confinement.

A central observable in our study is the tangent correlation function, \( G(s) \), that provides crucial conformational information about the filament’s microstructure. More specifically, it is defined as

\[ G(s) = \left\langle \cos \phi(s) \right\rangle, \tag{1} \]

where \( \phi(s) \) is the tangent angle of the filament at the arc length position \( s \), see sketch in Fig. 1(b). \( \left\langle \ldots \right\rangle \) in Eq. 1 denotes thermal average and \( \left[ \ldots \right]\) the spatial average along the contour length \( L \), i.e. \( \left[\phi(s)\right] = \frac{1}{L} \int_{0}^{L} ds \int_{0}^{s} du f(s-u) \phi(u+s) - \phi(u) \), see also Fig. 1(b). Revisited (unpublished) experimental data on vimentin filaments whose set up is described in detail elsewhere \cite{12} are displayed in Fig. 2. Quasi two-dimensional microfluidic channels of height \( h \) and varying lateral confinement \( d \) were employed to confine these filaments. Upon limiting the thermal average of \( \cos \phi(s) \) to an individual filament, striking oscillations in \( G(s) \) set in, see Fig. 2. At prescribed height (\( h = 0.45 \mu m \)), the two profiles in the main body of figure 2 (red and yellow...
FIG. 1: (a) Left panel: Vimentin filament confined in a slit occasionally coiled up in a transient ring. Right panel: Model three dimensional helical filament getting confined to a plane forming a ring as an energy minimum, see Eq. (2) and text. (b) Upper panel: Typical oscillatory shape of an intermediate filament in a quasi two-dimensional microfluidic channel characterized by a lateral confinement \(d\) \([10]\). Lower panel: Geometrical setup of the model filament. The tangent vector \(t\) evolves with arc-length \(s\) along the filament. Its direction is given by the angle \(\phi(s)\), measured from the horizontal. \(\psi(s)\) denotes the twist angle represented by a black ribbon on the filament’s surface. The variation of the twist at curvature inversion points gives rise to conformational defects called twist-kinks (TK).

symbols) correspond to two different lateral confinements (\(d = 1.6\) µm and \(d = 2.7\) µm). \(G(s)\) increases with decreasing \(d\) (i.e., with increasing degree of lateral confinement) as expected, whereas the oscillation amplitudes as well as their associated wave length decrease. These features remain robust upon changing the channel height to 1 µm, see green circles inset of Fig. 2. It is also possible to observe even stronger and more persistent oscillations, although such conformations are rather rare events, see data with blue circles in inset of Fig. 2.

In the following, we are going to rationalize these experimental observations by means of Monte Carlo (MC) simulations as well as analytical theory. The underlying idea is that confined IFs can be modeled as a helical superstructure trapped on a flat surface and subject to an additional lateral confinement.

Consider a semi-flexible helical filament of length \(L\) becoming confined to a plane. Its elastic energy is given by \([8, 9]\)

\[
E = \frac{1}{2} \int_0^L \left[ B \left( \phi' - \omega_1 \sin \psi \right)^2 + C \left( \psi' - \omega_3 \right)^2 + B\omega_1^2 \cos^2 \psi \right] ds ,
\]

where \(\phi'(s) =: \kappa(s)\) stands for the curvature, \(\psi\) designates the twist angle and \(\psi'(s)\) the twist with \(s\) being the arc-length, see also Fig. 1(b). The constants \(B\) and \(C\) in Eq. (2) are the bending and torsional stiffness, respectively, \(\omega_1\) and \(\omega_3\) are the preferred curvature and twist of the unconfined three-dimensional helical filament \([12]\). The filament ground state stemming from Eq. 2 obeys two coupled equations: The pendulum-like equation (i) \(\psi'' + \frac{B\omega_1^2}{Bc^2} \sin(2\psi) = 0\) and (ii) \(\kappa = \omega_1 \sin \psi\) indicating that curvature is slaved by the twist angle in contrast to the unconfined three-dimensional case (where both decouple). In general, depending on the material parameters, a rich variety of equilibrium shapes resembling loops, waves, spirals or circles exist \([9]\). As a matter of fact, these shapes can be seen as the result of interacting repulsive conformational defects corresponding to curvature inversion points. In terms of twist, such defects originate from a rapid variation of \(\psi(s)\) (reminiscent of a kink) and are called twist-kinks (TKs). A relevant di-
Our best matching MC data for \( G(s) \) (not shown) clearly indicates that the average number of twist-kinks \( n \) also describes the number of extrema encountered in \( G(s) \). Thermal fluctuations induce injections and ejections of twist-kinks at the ends of the filament, which qualitatively explains the fading of the oscillations in \( G(s) \) shown in Fig. 2. Concomitantly, a finite temperature induces a broadening in \( p(n) \).

To shed more light on the underlying physical mechanisms of confinement-mediated twist-kink production let us consider a more tractable analytical model. It consists in replacing the confinement by a pulling force at zero temperature, see Fig. 4. Although this systems is different it illustrates the twist-kink injection mechanism in a phenomenological manner. In the presence of the external force \( F \) and for small deformations \((\varphi \ll 1)\) the elastic energy becomes

\[
E_F \simeq E + \frac{F}{2} \int_0^L \phi^2(s) ds.
\]

Consider the regime \( \gamma \gg 1 \) which has as a ground state (in absence of a force) a circular arc \( \phi(s) = -\omega_1 s \) and energy \( E_0 = C L \omega_1^2 / 2 \), see Fig. 4. The formation of a twist-kink is energetically favorable when the pulling force exceeds a critical value \( F_c = \frac{64}{\sqrt{BC}} \). The latter is the result of comparison of the elastic energy of a twist-kink-free filament \( (E_0 + \frac{\omega_1^2}{2} L^3 F) \) with that of a filament containing a single twist-kink located at the midpoint \( (E_0 + \omega_1 \sqrt{BC} + \frac{\omega_1^2 L^3}{2} F) \), see Fig. 4. In the opposite, small force regime \((F < F_c)\), the filament deforms like a stretched elastic arc, see Fig. 4.

\[
\begin{align*}
F &< F_c \\
F &= F_c \\
F &> F_c
\end{align*}
\]
Above $F_c$, one or more twist-kinks are formed, see Fig. 4. The required force to nucleate $n$ twist-kinks is given by $F_c(n) = \frac{48\sqrt{3}YR}{L^2\kappa^2}n^2(n+1)^2$. Note, that the kinks mutually repel each other giving rise to an ordered one-dimensional crystal-like structure of $n$ twist-kinks separated by circular arcs of switching curvature, see Fig. 4. Expanding the chain conformation in Fourier modes, and keeping only the dominant one we extract the tangent correlation function:

$$G(s) \approx 1 - \frac{16\ell^2\omega^2}{\pi^4} \sin^2\left(\frac{ps}{2\ell}\right) \left(1 + \frac{\ell\sin(\pi s/\ell)}{\pi(L-s)}\right) \quad (4)$$

with $\ell = L/(n+1)$ representing the distance between two adjacent twist-kinks. Typical profiles of $G(s)$, displaying oscillations similar to the confinement case, are shown in Fig. 3. Simulation results with external force, not shown here, corroborate these findings.

Thus experimental and theoretical results strongly suggest that vimentin filaments have a helical superstructure, indicated by remarkable oscillations in the tangent correlation function in confined geometry.

The molecular origin of the helical shape of vimentin filaments is unclear at this point. Oscillations in the tangent correlation functions of individual actin filaments were also previously observed and helical superstructures of microtubule reported. Thus, the question arises which general physical mechanism could be at the origin of the curved/helical shapes of these biofilaments. Here we propose a mechanism based on self-buckling, for which we suggest the name autocoiling, see Fig. 5. It is a generic feature of filaments exposed to surface stresses that induce a broken symmetry and thus curved states. It can be witnessed in the mundane example of a drying spaghetti, see Fig. 5(c). The outer layer of the spaghetti dries and shrinks faster and induces a buckling stress on the (transiently) more swollen core.

For vimentin, there are a few hints towards a surface stress from its molecular structure. During the assembly of its constituent monomers the monomers form highly elongated coiled-coil dimers, then tetramers and at an intermediate stage give rise to an approximately “spindle shaped” 32-mer (see images in Ref. [21])—the so called unit length filament (ULF) [22]. These ULFs then assemble into long filaments. The initially rugged, ”rough” filaments of spindle-like ULF subunits, longitudinally anneal and the filaments undergo a maturation phase during which the surface smoothens Ref. [21, 22]. We suggest that the spindle shape and the finite lateral thickness of the ULF can be understood as originating from double-twist-frustration caused by chiral (cholesteric) interactions of the coiled-coil alpha helices aligned along the axis. As proposed by Grason and Bruinsma [23], a bundle of chiral objects displays a finite size as the chains on the outside are progressively more tilted than those on the inside. This tilt causes a surface stress and axial shortening of the outer chains with respect to the inner ones. As the simplest possible model for self-buckling under a surface stress we consider an isotropic incompressible elastic rod of radius $R$ and length $L$. Its energy is $U = \frac{1}{2} \int dV \sigma_{ij}\varepsilon_{ij} + \lambda\Delta S$ where $\sigma_{ij}$ and $\varepsilon_{ij}$ are the bulk stress and strain tensor respectively. The constant $\lambda > 0$ is an isotropic tensile surface stress (surface energy density). $\Delta S$ denotes the variation of the surface after deformation. When negative, the term $\lambda\Delta S$ can compete with the positive elastic bulk energy.

Under a pure bending deformation with a constant curvature $\kappa$ of the rod, the cross-section is slightly deformed and $\Delta S < 0$ as deduced from the deformation field of a bent rod [24]. It can be shown that $\frac{\Delta S}{\lambda} \approx -\frac{1}{16}R^2\kappa^2$ where $S_0 = 2\pi LR$ is the surface of the undeformed rod. The total surface+elastic energy can then be written as $U = \frac{1}{2}B_e(\lambda)L\kappa^2$ with an effective bending modulus that has contributions from both the bulk and the surface stress of the rod: $B_e(\lambda) = \frac{Y}{4}R^4 - \lambda\frac{11}{24}R^3$ where $Y$ is the Young modulus. For sufficiently large $\lambda$ the stiffness $B_e(\lambda)$ vanishes and the rod becomes unstable. This is the signature of a spontaneous broken symmetry of the rod, i.e., self-buckling.

Beyond the curvature, the origin of helical torsion of vimentin, its magnitude and handedness deserve further theoretical and experimental investigations. At this point we speculate that the handedness and the pitch of IFs are inherited from the chirali inter-alpha-helix interaction on the monomer level.

In summary, we report experimental and theoretical evidence for a helical superstructure in intermediate filaments. Lateral confinement in a quasi-2D channel orders the otherwise soft and strongly fluctuating twist-kink defects and leads to experimentally detectable, oscillating tangent correlation functions. The underlying physical mechanism is the injection of low energy defects, that can be seen as a new deformation mode for confined helical filaments. This behavior is reminiscent of the plastic deformations mediated by dislocations in solids [16]. The self-buckling mechanism suggested to be at the origin of the helical states of vimentin could also be common in other biological filaments beyond IFs. More generally,

![Fig. 5](image)

FIG. 5: a) Stages of assembly of IFs and their autocoiling instability. b) The cross-section deformation of the bent circular rod. c) The autocoiling of drying spaghetti.
tensile surface stress could be induced by any mismatch between the surface and the bulk of the filament, like e.g. the surface tension between the ordered water and ions at the outer layers of the filament that display mismatched osmotic and Maxwell stresses between the interior and exterior.

The biological meaning of an intrinsically stiffer but coiled helical structure is apparent. A coiled structure resists small forces by uncoiling and displays high compliance without damage up to the point of stronger elongation. This reinforces the IFs’ natural role as supporting mechanical elements and protective stress absorbers of the cell.

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[25] The number of twist-kinks can fluctuate at a finite temperature. The translational zero mode of the injected and moving twist-kinks leads to a chain with anomalously large "hyperflexibility" [8]. A remarkable analogy can be established with dislocations in solids, where (plastic) deformation is facilitated by the production of such energetically cheap linear defects in crystals [19].
[26] Each monomer is characterized by four variables \((x_i, y_i, \phi_i, \psi_i)\) with \(i = 1 \ldots N\). The positions \(x_i = x_{i-1} + \cos \phi_{i-1} \) and \(y_i = y_{i-1} + \sin \phi_{i-1}\) stand for the Cartesian coordinates in the plane. One Monte Carlo step consists of \(N\) attempted local rotations and torsions of each monomer sequentially and a single attempt of a global translation (only relevant in the presence of the confining channel), rotation [13] and torsion. Standard Metropolis scheme is then used to generate a canonical ensemble [14]. Typically, \(10^8\) Monte Carlo steps were employed for a simulation run and about the second half of the steps was used to gather statistics.
[27] (i) For simulation data with finite twist, the elastic constants are \(B/(k_BT) \simeq 50 \mu m\) and \(C/(k_BT) \simeq 3 \mu m\) except for the corresponding experimental data with blue circles where \(C/(k_BT) \simeq 10 \mu m\). The geometrical parameters are typically \(\omega_1^{-1} \approx 2 \mu m\) and \(\omega_2^{-1} \approx 1 \mu m\) (ii) For twist-free filaments (i.e., \(\omega_1 = \omega_2 = C = 0\)), \(B/(k_BT) \approx 5 \mu m\).
[28] Note that the value of \(B\) is much higher than determined previously from multi-filament averaging - a procedure that neglects single filament correlations and measures an “apparent” persistence length.