Direct Observation of Biaxial Confinement of a Semiflexible Filament in a Channel

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Actin is a key component of the protein complex responsible for producing contractile force in skeletal muscle. It is highly enriched near the cell surface in nonmuscle cells and plays an important role in both a cell's shape deformations and response to mechanical stress.[1−4] Filamentous actin, called F-actin, is a two-stranded helical protofilament with a diameter of ~80 Å and a contour length of ~10 μm. The experimental results show that the persistence length L_p of F-actin is 4−20 μm.[5−8] One of interesting experiments is to find the statistics and behaviors of a semiflexible filament in a confined space, such as a channel width $W \leq L_p$,[9] which has in fact never been reported before. Because the persistence length of F-actin is long enough to be visualized in situ by video fluorescence microscopy, actin is an ideal system for this study.

The tangent-tangent correlation function $\langle C(s)\rangle$ is defined as

$$\langle C(s)\rangle = \langle \vec{u}(0)\cdot\vec{u}(s)\rangle = \langle \cos[\theta(s) - \theta(0)]\rangle \qquad (1)$$

where s is the arclength along the chain, $\vec{u}(s)$ the unit tangent vector along s, and θ the angle measured from the channel axes (see Figure 1a). The persistence length is the characteristic distance along the chain at which the unit vector $\vec{u}(s)$ becomes uncorrelated. For a semiflexible polymer in two-dimensional free space,

$$\langle C(s)\rangle \sim e^{-s/2L_p} \qquad (2)$$

The persistence length is intrinsic to the chain and is determined, for example, by buffer and polymerization conditions.[8] In particular, it is constant for an unconfined chain in two dimensions, independent of the contour length of the chain. For a polymer confined to a channel, however, this definition is not applicable because $\langle C(s)\rangle$ does not generically decay exponentially. In this letter, we ask whether we can define a new length scale, the effective persistence length L_p^{eff}, which is determined by the geometrical constraints on the chain configurations due to confinement.

Alexa-488-labeled globular (G-) actin was suspended in a standard buffer solution.[10] Polymerization was carried out mainly with 100 mM KCl and the addition of phalloidin to stabilize the filaments. Microchannels with a depth of 1 μm and widths of 3, 5, 10, and 20 μm, were fabricated on Si substrates using photolithography and reactive ion etching. To prevent the F-actin solution from evaporating, the channels were sealed with a water-insoluble oil, as shown in the cartoon Figure 1b. A single channel was filled with 2 μg/mL of F-actin solution through a glass capillary tube using a microinjector.[11−12]

To investigate the thermal fluctuations of a F-actin in a channel, we used a high-resolution video microscopy system, composed of an optical microscope and a silicon-intensified target (SIT) camera VE1000.[13] At room temperature, a single chain of F-actin undergoes fluctuations with characteristic time scales of seconds. The F-actin fluctuations were recorded using video microscopy and then were captured by a frame grabber. F-actin in the fluorescence images (Figure 1c) has a thickness of ~1 μm and a contour length of ~18 μm.

The confinement effect is not conspicuously observed in the channel widths of 20 and 10 μm. F-actin in a 20 μm channel undergoes random fluctuations consistent with unconfined F-actin in two dimensions, although F-actin in the 10 μm channel is occasionally deflected by the sidewalls of the channel and is flattened along the wall, as shown in Figure 1(c: second upper). In the 5 μm channel, however, this flattening effect is always observed. Figure 1(c: upper third) shows an S-shaped configuration of F-actin in the middle of transverse fluctuations. The confinement obviously affects F-actin configurations for $W \leq 5$ μm.

We introduce a simplified model to study the tangent−tangent correlation function of confined F-actin. We model the confined chains as two-dimensional wormlike chains, where the channel axis is in the $\hat{x}$ direction and $\hat{y}$ is the confined direction. We also assume that the chain is infinitely long and can be described by a function $\boldsymbol{r}(s)$, where s is the arclength along the chain. Further, we assume that the chain is confined by a harmonic potential in the $\hat{y}$ direction with a spring constant k. The partition function is

$$\mathrm{Z} = \int \mathbf{D}\vec{r}\,\delta[(\partial_s\vec{r})^2 - 1]\, e^{-\beta\int \mathrm{d}s[1/2\kappa_0(\partial_s^2\vec{r})^2 + 1/2k(r_y)^2]} \qquad (3)$$

where $\beta \equiv 1/k_BT$ and the bending modulus $\kappa_0 \equiv k_BTL_p$.

In the high confinement limit, $\sin\theta \approx \theta$,[14] where θ measures the angle from the x axis, and the model is completely solvable. The tangent−tangent correlation function is given by

$$\ln\langle C(s)\rangle \approx \frac{k_BT}{2(k\kappa_0^3)^{1/4}}\, e\frac{|s|}{\sqrt{2}}\left(\frac{k}{\kappa_0}\right)^{1/4} \left\{\cos\left[\frac{|s|}{\sqrt{2}}\left(\frac{k}{\kappa_0}\right)^{1/4}\right] - \sin\left[\frac{|s|}{\sqrt{2}}\left(\frac{k}{\kappa_0}\right)^{1/4}\right]\right\} - \frac{k_BT}{2(k\kappa_0^3)^{1/4}} \qquad (4)$$

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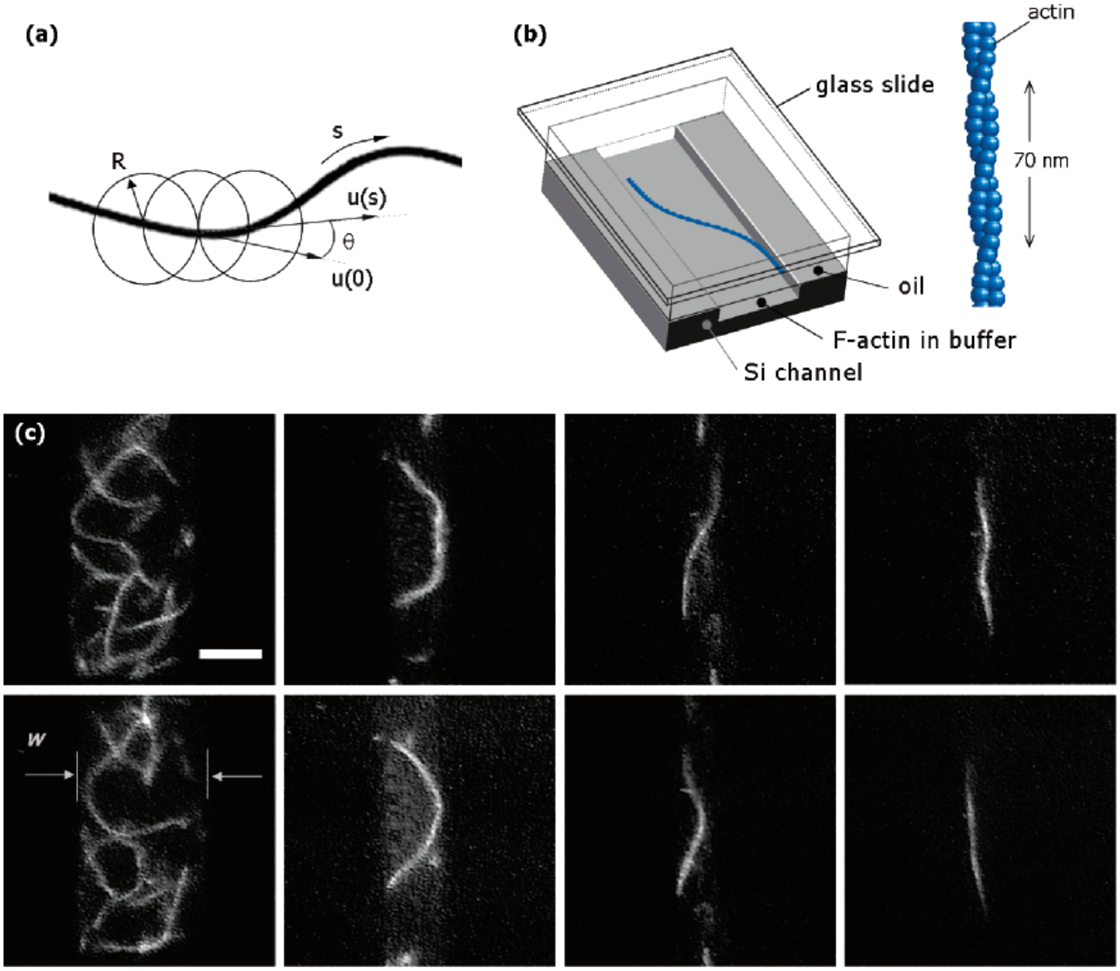

Figure 1. (a) Measurement of the tangent−tangent correlation function. (b) Graphic illustration of F-actin confined in microchannel. (c) Fluorescence video microscopy images of F-actin fluctuations in microchannels: from left to right, the channels are 20, 10, 5, and 3 μm wide, respectively, and 1 μm deep. Top and bottom images were taken in arbitrary time. Scale bar indicates 10 μm.

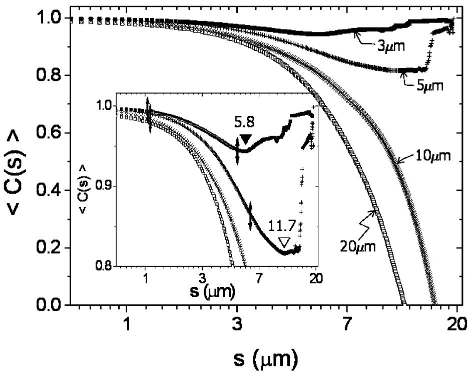

Figure 2. $\langle C(s)\rangle$ for $W = 3$ μm (■), 5 μm (+), 10 μm (×), and 20 μm (□). (Inset) L_p^{eff} were extracted by fitting the correlation curves to eq 2. Two cases, $W = 3$ and 5 μm, are represented as examples. The double arrowed bars indicate the fitting region s_{fit}. In the 3 μm channel, for example, 1 μm ≤ s_{fit} ≤ 5 μm, where 1 μm is the optical resolution limit of the fluorescence measurement.

and the effective channel width W can be defined by $W^2 \equiv \langle r_y(0) r_y(0)\rangle = k_B T/2(k^3\kappa_0)^{1/4}$. The first minimum of the tangent−tangent correlation function in terms of W is

$$s_{\min} = \frac{\pi}{\sqrt{2}} W^{2/3} L_p^{1/3} \qquad (5)$$

where L_p is the persistence length of the chain in two-dimensional free space. Additional minima in eq 4 are strongly suppressed. For the purpose of comparison with experiments, we take W to be the width of the microchannel.

The minimum, $s_{\min}$, has a natural interpretation as the contour distance between deflections of the filament due to the walls.[9] At long distances, $\ln\langle C(s)\rangle \rightarrow (L_p/W)^{2/3}$, signifying the presence of long-range orientational order induced by the confinement. Unfortunately, the F-actin appears too short to see this effect.

We measured $\langle C(s)\rangle$ from the fluorescence images for four different channel widths (Figure 2). Two correlation curves of $W = 20$ and 10 μm fall rapidly to zero, but those of the 3 and 5 μm channels never decrease below $\langle C(s)\rangle = 0.9$. Instead, they rise again, but fail to return to $\langle C(s)\rangle = 1$.

Equation 4 becomes $\ln\langle C(s)\rangle \approx -s/2L_p + O(s^2)$ for $s \ll s_{\min}$. To obtain the experimental persistence length of the chain, we fit the decaying portion of the experimental data to $\langle C(s)\rangle \sim e^{-s/2L_p^{\mathrm{eff}}}$ as shown in Figure 2(b: inset). The measured values of L_p^{eff} are 5.9, 10.0, 29.3, and 38.2 μm for $W = 20$, 10, 5, and 3 μm, respectively. $L_p^{\mathrm{eff}} = 5.9$ μm at $W = 20$ μm is similar to the persistence length measured in two-dimensional free space in other experiments,[5,7−8] that is, $L_{p\,(W\,=\,20\mu m)}^{\mathrm{eff}} \approx L_p$ in this study.

According to eq 5, for a semiflexible polymer under confinement, there exists a point $s_{\min}$ at which the tangent–tangent correlation function attains a minimum. The theory gives the values of $s_{\min} = 8.4$ and 11.7 μm for $W = 3$ and 5 μm, respectively, using the bare persistence length $L_p = 5.9$ μm to determine κ_0. In comparison, we experimentally measured $s_{\min}$ from the correlation curves of Figure 2 and found $s_{\min} = 5.8$ and 11.7 μm for $W = 3$ and 5 μm (Figure 2 (inset)). However, there is no minimum in the case of the channels of width $W = 10$ and 20 μm. We find good agreement between theory and experiment for $W = 5$ μm, but not for $W = 3$ μm. The reason for the inconsistency for the 3 μm channel is not yet clear, but one possibility is that the assumption of a two-dimensional polymer is increasingly inaccurate with decreasing channel width due to the effect of the channel depth. In particular, if d is the depth of the channel, it is necessary that $d \ll W$ for the theory to be valid.

In summary, we presented the first direct observation of the biaxial confinement of F-actin by a microchannel. With decreasing channel width, F-actin undergoes a transition from a 2D randomly oriented regime to a 1D biaxially confined regime with increasing L_p^{eff}. We found that the tangent–tangent correlation function of a semiflexible filament in a confinement regime shows a minimum, $s_{\min} = \pi W^{2/3} L_p^{1/3} \sqrt{2}$ with $W \le L_p \le s_{\min} < L_p^{\text{eff}}$, consistent with experiment.

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Supporting Information Available: Details of calculation of the tangent–tangent correlation function for wormlike chains in two dimensions confined by a harmonic potential. Materials and experiment information on cleaning of Si microchannels, microinjector system InjectMan and Transjection 5346 (Eppendorf) mounted onto optical microscope (Nikon upright type Microphot-FX), and fluorescence microscopy system. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) G-actin, Alexa-Fluor 488 conjugate, was purchased from Molecular Probes Inc., Eugene, OR.; G-actin buffer, with 5 mM Tris-HCl, 0.2 mM $CaCl_2$, 0.5 mM ATP, and 0.2 mM DTT, was purchased from Cytoskeleton Inc., Denver, CO.

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(13) SIT camera purchased from Dage-MTI Inc., Michigan, IN.

(14) We assume that the angle between the tangent vector of F-actin and the sidewall is small. To verify this, the angular distributions were measured. The distributions fit well with a Gaussian curve centered at 0°, −6.5°, with its fwhm at 16°, 19°, respectively, for $W = 3$ and 5 μm. It can be interpreted that F-actin does not bend more than 8° (20°) from the sidewall of the 3 μm (5 μm) channel. However, in an unconfined regime (i.e., $W \ge 10$ μm), F-actin has a broad angular distribution between −70° and 50°.

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