Actin-binding proteins sensitively mediate F-actin bundle stiffness

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Filamentous actin (F-actin) bundles form primary structural components of a broad range of cytoskeletal processes including filopodia, sensory hair cell bristles, and microvilli. In vivo, F-actin is bundled by multiple actin-binding proteins (ABPs) that enable the cell to tailor bundle dimensions and mechanical properties to suit diverse biological functions including cell motility, phagocytosis, fertilization, and mechanosensation. Despite the importance of F-actin bundle mechanical properties to many cytoskeletal processes, quantitative investigations of their mediation by actin binding proteins (ABPs) remain scarce. We directly measure the bending stiffness of F-actin crosslinked by three ABPs that are ubiquitous in eukaryotes: I-plastin, fascin-1, and α-actinin and observe distinct regimes of bundle bending stiffness that differ by orders of magnitude depending on ABP type, concentration, and bundle size. The reported behaviour is reproduced quantitatively by a molecular-based mechanical model in which ABP shearing competes with F-actin extension/compression. A generic scaling parameter identifying the relevant bundle bending stiffness regimes is presented. Our results shed new light on the potential role of ABPs in associated cellular processes and demonstrate how single molecule properties determine mesoscopic material properties. Moreover, the observed mechanics of fiber bundle bending are completely general, with broad implications not only for cytoskeletal mechanics but also for the rational design of functional materials.

The mechanical requirements of F-actin bundles are intimately connected to the specific biological function that they are designed to fulfill. Bundles integrated into the cytoskeleton are designed principally to reinforce the cell against mechanical deformation, highly dynamic filopodia present at the leading edge of motile cells are needed to exert potentially large protrusive forces against the advancing membrane during phagocytosis or
migration through the dense extracellular space\textsuperscript{1-3}, while microvilli are passive structural elements that serve primarily to increase the apical surface area of intestinal epithelial cells, thereby enhancing diffusive nutrient transport. In each case, the cell uses specific ABPs to bundle F-actin and carry out its biological function. $\alpha$-actinin predominates in cytoskeletal bundles and muscle\textsuperscript{4}, fascin is prevalent in filopodia\textsuperscript{5,6}, and plastin is predominant in microvilli and stereocilia\textsuperscript{7,8}. The disparate mechanical requirements of these cellular processes, together with the broad evolutionary conservation of their predominant ABPs across vertebrate and invertebrate eukaryotes, suggests that a key component of ABP biological function is its ability to differentially mediate F-actin bundle stiffness\textsuperscript{9}. A mechanistic understanding of the effects of ABPs on F-actin bundle mechanics might thus be needed to obtain a complete understanding of their physiological role in cells\textsuperscript{10-12}. Quantifying the governing mechanical principles of these fundamental cytoskeletal constituents should also prove valuable in the design of biomimetic nanomaterials\textsuperscript{13}.

F-actin bundles can be characterized mechanically by their intrinsic bending stiffness, $\kappa_b$, which is related to their persistence length, $l_p$, in the same manner as for any polymer,

$$\kappa_b = l_p k_B T.$$ 

Here, $l_p$ is the length scale over which associated thermal fluctuations destroy memory of a direction initially tangent to the bundle backbone and $k_B T$ denotes thermal energy, where $k_B$ is the Boltzmann constant and $T$ is absolute temperature. $\kappa_b$ is the mechanical property of interest for F-actin bundles because it can be used to calculate, for example, the maximal force that can be exerted by filopodia prior to buckling\textsuperscript{2,3,14} or mechanotransduction mechanisms of brush-border microvilli\textsuperscript{15} and hair cell stereocilia\textsuperscript{16,17}.

Two limiting types of F-actin bundle bending with notably different associated $\kappa_b$ have been reported (Fig. 1)—\textit{decoupled bending}, in which constituent actin filaments bend independently because intervening crosslinks do not resist shear and instead “tilt” freely during bundle bending\textsuperscript{18,19} and \textit{fully coupled bending}, in which filaments are rigidly “glued”
together by intervening crosslinks that strongly resist shear, forcing filaments away from the
bundle neutral surface and force them to stretch or compress during bending. The former
scenario results in a simple linear dependence of $\kappa_B$ on the number of filaments, $n$,
constituting the bundle. This scaling is what one expects when bending a loose stack of paper
and has been observed in the sensory hair bundles of the frog sacculus, predominant in the
ABP plastin$^{18}$. In contrast, the latter scenario results in a much stronger quadratic
dependence, $\kappa_B \sim n^2$, identical to the result for a standard homogeneous mechanical beam, as
one would expect if the sheets of paper were glued rigidly together to prohibit interlayer shear
deforation. This fully coupled regime was measured quantitatively for the crystalline actin
bundle found in the acrosomal process of horseshoe crab sperm cells at fully saturated
scrin:actin concentrations$^{20}$. Thus, ABP type clearly affects the degree of shear coupling
between F-actin filaments and consequently $\kappa_B$. However, it is not obvious a priori what role
specific molecular properties such as ABP shear and extensional stiffness, molecular length,
and concentration play in mediating the associated bundle bending regime.

To address these questions, we systematically investigate the dependence of $\kappa_B$ on
bundle size and ABP type and concentration using an emulsion droplet system$^{21}$. Briefly,
actin is polymerized in the water phase of a water-dodecane emulsion stabilized by
phospholipids. In the absence of bundling proteins, this procedure results in isolated emulsion
droplets containing entangled F-actin solutions that can be observed directly using
fluorescence microscopy (Fig. 2a). Inclusion of ABPs such as fascin, plastin, or $\alpha$-actinin in
the polymerization process results in a single compact F-actin bundle formed into a closed
ring (Fig 2b). As demonstrated theoretically by Odijk$^{22}$, the amplitude of the transverse
thermal fluctuations of the ring backbone is determined at any given temperature solely by $\kappa_B$
and the radius of the ring, $R_r$ (Fig. 2b and Methods). Thus, systematic variation of droplet
volume and actin-ABP concentrations in the microemulsion system enables for the first time the controlled investigation of the dependence of $\kappa_B$ on $n$ for each ABP considered (Fig. 2).

For each ABP type and concentration examined, $\kappa_B$ depends strongly on $n$ and converges to the expected single filament value of, $7 \times 10^{-26}$ Nm$^2$ ($l_p = 17 \times 10^{-6}$ m at $T = 298$ K) (Fig. 3a–d). Differences in the dependencies of $\kappa_B$ on $n$ for the different ABP types and concentrations, $c_{ABP}$, are however drastic. The $\kappa_B$ of plastin-crosslinked bundles increases linearly with $n$ independently of $c_{plastin}$ (for $c_{plastin} / c_a \leq 0.5$) (Fig 3a), whereas the $\kappa_B$ of fascin-crosslinked bundles depends strongly on both $n$ and $c_{fascin}$ (Fig. 3b).

The linear scaling exhibited by $\kappa_B$ for plastin-crosslinked bundles is consistent with the decoupled bending scenario in which F-actin filaments contribute equally and independently to $\kappa_B$ (Fig. 1), even at high $c_{plastin} : c_a$. We postulate that the physical origin of this observation is that plastin is too weak to resist the inter-filament shear deformation associated with decoupled bundle bending, which is consistent with the crosslink tilting observed in plastin-F-actin bundles using EM$^{19,24}$. Additional support for this hypothesis is provided by the dependence of $\kappa_B$ on $n$ and $c_{fascin}$, which is quantitatively explained by a purely mechanical model of fiber bundle bending that accounts for the interplay between F-actin filament stretching and inter-filament ABP shearing$^{25}$ (Methods).

In this mechanical model, F-actin is treated as a standard Euler–Bernoulli beam that is capable of extension/compression in addition to pure bending$^{25}$ (Fig. 1 and Methods). Crosslinking ABPs mechanically couple neighboring filaments during bundle bending by resisting inter-filament shear with shear stiffness, $k_{\parallel}$. ABP-actin binding affinity and $c_{ABP}$ in the droplet determine the mean axial spacing of ABPs in the bundle, $\delta$, via chemical equilibrium, which gives rise to an overall effective shear stiffness coupling neighboring filaments, $k_{\parallel} / \delta$. The single unknown parameter in the model, $k_{\parallel}$, is an intrinsic property of
any ABP that is a result of protein structure and binding geometry. The bending stiffness of fascin-crosslinked F-actin filaments can be fitted uniquely as a function of both \( n \) and \( c_{\text{fascin}} \) using \( k_{||} = 1.5 \times 10^{-5} \) N/m (Fig. 2b). The plasmin-concentration-independence of \( \kappa_{B} \) allows only an upper bound of \( k_{||} \leq 0.05 \times 10^{-5} \) N/m to be calculated.

The crossover in \( \kappa_{B} \) from \( n^2 \)- to \( n \)-scaling has its origin in the finite shear stiffness of the crosslinks. The mechanical model predicts that \( \kappa_{B} \) must eventually crossover from \( n^2 \) to \( n \)-scaling with increasing \( n \) due to a competition between crosslink shearing and F-actin filament stretching/compression. Indeed, agreement between the model and experimental data for fascin suggests that this crossover is observed at all but the lowest concentration examined, where a fully coupled \( n^2 \)-scaling at low \( n \) is superseded by linear scaling at higher \( n \) (Fig. 3b). This crossover originates from the fact that in order to maintain \( n^2 \)-scaling, each filament that is successively added to the outer shell of the bundle must be extended/compressed proportionately because the axial strain field in a fully coupled bundle varies linearly across the cross-section. The linearly increasing energetic cost of adding filaments to the bundle in this manner must therefore eventually outweigh the cost of shearing the crosslinks instead, which is independent of bundle size. At that point, crosslink shearing relieves filament stretching/compression, leading directly to the crossover in \( \kappa_{B} \) from fully coupled \( n^2 \)- to \( n \)-scaling. This regime is shear-dominated and intermediate to the fully coupled and decoupled regimes. The fact that the crossover in \( \kappa_{B} \) occurs at decreasing \( n \) with decreasing fascin concentration (Fig. 3b) is consistent with this picture because it is the effective shear stiffness, \( k_{||} / \delta \), that denotes the strength of the shear coupling. A scaling analysis that evaluates the energetic costs of shearing and bending suggests that the dimensionless quantity, \( \alpha \equiv k_{||} L_{B}^2 / \delta k_{a} \), denotes the relevant bundle bending regime, where \( (\alpha \gg n) \), \( (\alpha \ll 1) \), and \( (1 \ll \alpha \ll n) \) for the fully coupled, decoupled, and shear-dominated
regimes, respectively. $L_B$ is the bundle length and $k_a = 4.4 \times 10^{-8} \text{N}$ is the axial stretching stiffness of F-actin\textsuperscript{26}. This scaling result is generally applicable to any crosslinked fiber bundle provided that $k_a$ is substituted with the appropriate stretching stiffness of the constituent fiber.

Increasing the length of the crosslinker shifts the crossover in $\kappa_B$ to lower $n$ because the energetic cost associated with filament stretching/compression in fully coupled bending is proportional to the distance of the filament to the center of the bundle. This effect is observed for $\alpha$-actinin (Fig 3c), which has a molecular length that is roughly three times that of fascin. As a result, the crossover occurs at only $n \approx 3–4$ and a relatively large associated shift in $\kappa_B$ is observed. Fitting the model to the data yields, $k_\parallel = 10^{-5} \text{N/m}$.

Depletion forces induced by small molecules present in the cytosol may also act as effective crosslinkers that bundle F-actin, as demonstrated \textit{in vitro} using polyethylene glycol (PEG)\textsuperscript{27,28}. To examine the potential role of depletion forces on F-actin bundle stiffness, we also measured $\kappa_B$ in the presence of PEG (MW 6 kDa, 2 \& 4% w/w). Interestingly, $\kappa_B$ depends \textit{quadratically} on $n$ for the entire range of $n$ examined (Fig. 3d), indicating that the bundle remains in the \textit{fully coupled} regime for all bundle sizes investigated ($n < 30$). This places a lower bound on PEG’s \textit{effective} crosslinking shear stiffness per unit length of, $k_\parallel / \delta \geq 10^3 \text{N/m}^2$. This value can be compared directly to the ABPs examined assuming, $\delta = 10^{-7} \text{m}$, the typical ABP axial spacing at high ABP concentration, which results in, $k_\parallel \geq 10^{-4} \text{N/m}$. We postulate that this relatively high stiffness has its origin in a tight inter-molecular packing of the helical F-actin filaments that prohibits inter-filament slip. Given that depletion forces induce fully coupled bundle bending, an important functional role of ABPs \textit{in vivo} might actually be to prevent fully coupled bending by acting as inter-filament spacers.
Folded proteins have complex, anisotropic mechanical properties that depend strongly on secondary and tertiary structure, the magnitude of applied deformations, and the length scales probed\textsuperscript{29}. In our experiments thermal fluctuations of the bundle backbone result in ABP shear strains on the order of only one percent. Thus, ABPs are deformed on only the Ångstrom-scale and the observed shear stiffness may be expected to be considerably lower than the extensional stiffness probed by single-molecule unfolding experiments ($10^{-3} – 10^0$ N/m) typically with nanometer-resolution\textsuperscript{30,31}. The hierarchical structure of proteins allows for a nonlinear mechanical response: a soft response at small deformations is possible without compromising protein stability. Detailed molecular simulations are required to elucidate the precise origin of the observed ABP shear stiffness.

Living cells employ a limited number of ABPs to tightly crosslink F-actin filaments into bundles, and a single ABP type can predominate a given cytoskeletal process. The values of ABP shear stiffness observed here can be used together with the known lengths of physiological bundles to determine the dimensionless parameter, $\alpha$, and thus the associated bending regime for F-actin bundles found in nature. The \textit{decoupled} bending regime clearly indicates a mechanical preference for maximal bundle compliance, the \textit{fully coupled} regime for maximal bundle stiffness, whereas the \textit{shear-dominated} regime could indicate a preference for an actively tunable bundle stiffness that may be varied between the two limits by varying ABP concentration or bundle size. For example, brush border microvilli are predominant in plastin, range from 2–5 µm in length, and consist of 20–30 filaments. Thus they are in the decoupled bending regime, which could have important biological consequences. Interestingly, due to the typically observed lengths of filopodia, even their bending response is expected to be in this regime. However, it is re-emphasized that we only explore the linear, small deformation shear response of ABPs in this work and nonlinear ABP shear response may be important. A biological advantage of the observed soft shear stiffness of ABPs could be that distinct mechanical response regimes can be fully exploited depending
on the magnitude of bundle deformations. The hierarchical stiffness of proteins and their
highly nonlinear response provides an additional means of tuning the bending stiffness of F-
actin bundles. It remains a formidable challenge to explore the nonlinear mechanical response
of such bundle structures.

Finally, the results presented here highlight the importance of using in vitro systems to
determine the biomechanical function of ABPs especially considering practical difficulties
associated with in vivo experiments. Comparing the mechanical properties of bundles formed
using a range of ABPs, we demonstrate that bundle mechanics are defined by single molecule
properties. It is the formidable challenge of understanding the relation between single
molecule structure, mechanics, and the collective behavior of their macromolecular
assemblies that is necessary to bridge the existing gap in our understanding of biologically-
important processes\textsuperscript{10-12}. The governing principles of bundle-bending elucidated in this
investigation are completely general and equally applicable to fiber bundles of microtubules
or carbon nanotubes as they are to bundles of F-actin. We isolated the dimensionless bundle
bending stiffness parameter, $\alpha$, which may be used in the future to rationally design biological
and biomimetic (nano-)materials that employ fiber bundles for enhanced structural stiffness,
by tuning the associated bundle bending regime and hence the associated bending stiffness by
orders of magnitude.
Methods

Protein preparation. α-actinin is isolated from turkey gizzard smooth muscle\textsuperscript{32}, dialyzed against G-buffer (2 mM Tris, 0.2 mM ATP, 0.2 mM CaCl\textsubscript{2}, 0.2 mM DTT, 0.005% NaN\textsubscript{3}) and stored at 4 °C for several weeks. Recombinant plasmids containing either the full length human I-plastin cDNA (kind gift from F. Rivero, Köln, Germany) or human fascin (kind gift of D. Vignjevic, Paris, France) are transformed in \textit{Escherichia coli} L12-codon+ bacteria. \textit{E. coli} bacteria carrying the plasmid are grown at 37 °C until the $A_{600}$ reached 0.6. Protein expression is induced with isopropyl-β-D-thiogalactopyranoside (IPTG) at 37 °C for I-plastin and at 20°C for fascin. G-actin is extracted from rabbit skeletal muscle according to\textsuperscript{33}. G-actin solutions are prepared by dissolving lyophilized G-actin in deionized water and dialyzing against fresh G-buffer at 4°C for 24 hours. Solutions of G-actin are kept at 4°C and used within 7 days after preparation. G-actin is polymerized in the presence of ABP and phalloidin TRITC (Sigma)(1:4) inside the water phase of a water-in-dodecane emulsion\textsuperscript{21}. Actin polymerization is initiated by adding 1/10th of the sample volume of a 10-fold concentrated F-buffer (20 mM Tris, 20 mM MgCl\textsubscript{2}, 2 mM CaCl\textsubscript{2}, 2 mM DTT, and 1 M KCl). After gentle mixing, 20 µl of this solution is immediately transferred to 1 ml of a 0.3 mM phospholipid-containing (95 mol % DOPG, 5 mol % DMPE-PEG2000 (Avanti Polar Lipids)) dodecane solution. Emulsion droplets are subsequently prepared by vortexing the solution for a short time. The negatively charged phospholipids stabilize the droplets against coagulation and mimic the presence of a fluid membrane with essentially no bending undulations. The addition of DMPE-PEG2000 prevented adsorption of filaments and ABPs to the droplet wall. Recombinant I-plastin is reported to bundle actin filaments in the absence, and not in the presence, of calcium. Accordingly, calcium-free actin preparation and F-buffer are used for I-plastin experiments.
Instruments and processing. All data are acquired on a Zeiss Axiovert 200 inverted microscope with a 100x/1.3 NA oil objective. Images are captured at a frame rate of one image per 117 milliseconds with a CCD camera (C4880-80, Hamamatsu) and stored on hard disk. Image storage and analysis are performed with the image processing software “OpenBox”\textsuperscript{34}. Samples are maintained in a fluid chamber. Silanization of the glass surface with dichlorodimethylsilane (Sigma) prevented adsorption of the emulsion droplets. To measure the “fuzzy diameter,” $D_B$, of an F-actin bundle in a ring geometry, the fluorescence intensity profile along a line perpendicular to the bundle is followed in time. Fluctuations are followed for 5–12 seconds and the intensity profiles are summed, the width of the distribution is independent of the sampling time, indicating full equilibration. The wall of the droplet merely fixes the center of mass of the ring and does not affect the transverse fluctuations of the bundle. In-plane and out-of-plane transverse fluctuations are equal, confirming that the bundle bending stiffness is isotropic (see Theory). Gaussian distributions are fit to both the instantaneous and the time-averaged fluorescence intensity profiles of the bundle and the standard deviations, $\sigma_{\text{int}}$ and $\sigma_{\text{avg}}$, respectively, of the distributions are determined. The fuzzy diameter of the bundle is, $D_B = \sigma_{\text{avg}} - \sigma_{\text{int}}$, because $\sigma_{\text{int}}$ does not contribute to the fluctuation amplitude of the bundle. Bundle length $L_B$ was directly proportional to $n$ and the experimentally determined linear relation was used in the further analysis.

Theory. A F-actin bundle is assumed to behave like a worm-like chain (WLC) with bending stiffness, $\kappa_B$, and contour length $L_B$. The bending stiffness of a WLC bent into a ring geometry is related to the root-mean-square ring radius, $R_c$, by\textsuperscript{22}, $\kappa_B = 0.16R_c^2k_BT/D_B^2$, where $k_B$ is the Boltzmann constant, and $T$ is absolute temperature. Metropolis Monte Carlo simulations were used to determine the prefactor (0.16) and to confirm that WLCs with transversely isotropic bending stiffness have equal transverse fluctuations in- and out-of-the-plane of the ring. $D_B$ in- and out-of-the-plane of the ring are experimentally determined to be
equivalent, thereby confirming that the bundle bending stiffness is transversely isotropic. To determine the shear stiffness of the crosslinks, \( \kappa_B \) is calculated theoretically using a fiber-based bundle model\(^\text{25} \) (MB, EF in preparation 2006). The maximal deflection, \( w_{B,\text{max}} \), of a bundle subject to a unit load in three-point bending with pinned boundary conditions is calculated analytically using an analogous version of Eq. 11 of ref. 25 and equated with the equivalent displacement of a homogeneous Euler–Bernoulli beam, \( \kappa_B = L_B^3 / 48 w_{B,\text{max}} \), where,

\[
\begin{align*}
w_{B,\text{max}} &= \frac{L_B^3}{48 n \kappa_a} - \frac{k_c d (d + t) \sum_{i=1}^{N} i^2}{\delta K n^2 \kappa_a^2} \left[ \frac{L_B^3}{24 \beta^2} + \frac{L_B}{2 \beta^3} \tan \frac{\beta L}{2} \right] \\
\beta^2 &\equiv \frac{\frac{k_c}{\delta K \kappa_a} + \frac{2k_c d (d + t) \sum_{i=1}^{N} i^2}{\delta K n \kappa_a}}{\delta K n \kappa_a}
\end{align*}
\]

and \( K \approx \frac{1}{N} \sum_{k=1}^{N} \sum_{i=k}^{N} i \), where \( n \) is the total (odd) number of filaments in 2D and \( N \approx (n - 1)/2 \). F-actin fibers have stretching stiffness, \( k_a = EA = 4.4 \times 10^{-8} \text{ N}^2 \), and bending stiffness, \( \kappa_a = EI = 7.3 \times 10^{-26} \text{ Nm}^2 \), with diameter \( d = 4.5 \text{ nm} \) corresponding to an effective Young’s modulus of 2 GPa. The total number of fibers in this 2D theory is related to the number of fibers in 3D, by, \( n_{3D} = n_{2D}^2 \), and the corresponding 3D bundle bending stiffness is related to the 2D bundle bending stiffness by, \( \kappa_B(3D) = n_{(2D)} \kappa_B(2D) \). \( \delta \) is the axial spacing between crosslinks and \( t \) is the inter-filament spacing, taken to be 10 and 35 nm for fascin/plastin and \( \alpha \)-actinin, respectively\(^\text{24,36,37} \). \( \delta \) is calculated from ABP-actin chemical equilibrium using, \( \delta = 33 \times 10^{-9} / \theta \text{ m} \), where, \( \theta = c_{\text{ABP}} / (K_d + c_{\text{ABP}}) \), is the fraction of bound crosslink sites, \( K_d \) is the equilibrium dissociation constant for the relevant ABP binding to F-actin bundles (\( \alpha \)-actinin and plastin \( K_d = 0.5 \mu \text{M} \), fascin \( K_d = 0.7 \mu \text{M} \)), and \( c_{\text{ABP}} \) is the droplet ABP concentration, which is confirmed to be independent of finite-size effects. The minimum mean axial spacing between crosslinks, \( \delta_{\text{max}} = 33 \text{ nm} \), corresponds to hexagonally packed F-
actin bundles with fully saturated binding sites. The only unknown parameter in the model, \( k_b \), is used to fit the experimental \( \kappa_b \) data uniquely for each ABP crosslink examined.

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Competing interests. The authors declare that they have no competing interests.
References


Figure captions

Figure 1
F-actin bundle model. F-actin filaments (black) are coupled to nearest-neighbor filaments by discrete ABPs (green) with axial spacing, $\delta$ [m], and shear stiffness, $k_\gamma$ [N/m]. F-actin is characterized mechanically by its backbone extensional stiffness, $k_u = EA = 4.4 \times 10^{-8}$ N m, and bending stiffness, $\kappa_B = EI = 7.3 \times 10^{-26}$ N m$^2$. $E$ is the effective Young’s modulus, $A$ is the effective cross-sectional area, and $I$ is the second moment of the cross-sectional area of F-actin. The inter-filament spacing, $t$, is fixed by the length of the intervening ABPs and remains constant in tightly crosslinked bundles. The ratio, $\alpha = k_u L_a / \delta k_\gamma$, where $L_a$ is the bundle length, mediates a competition between crosslink shearing and F-actin filament extension or compression during bundle bending, which determines the bundle bending regime (see text for details).

Figure 2
Experimental setup. a, Isolated F-actin filaments polymerized in an emulsion droplet in the absence of ABPs form a random isotropic solution. b, Inclusion of ABPs in the polymerization process leads to the formation of a single F-actin bundle that organizes into a ring of root-mean-square radius, $R_r$. Actin concentration, $c_a$, and droplet volume, $V_d = 4\pi R_d^3 / 3$, where $R_d$ is the droplet radius, determine the total length of F-actin present in the droplet and thus the number of filaments, $n$, constituting the bundle. Thermal fluctuations of the bundle backbone are characterized by the fuzzy diameter, $D$, which is directly related to $\kappa_B$ (Methods). Scale bars are 10 $\mu$m.

Figure 3
F-actin bundle bending stiffness, $\kappa_B$. a, Bundles crosslinked by plastin exhibit linear scaling in the number of filaments, $n$, constituting the bundle for both low (○ 1:50) and high (● 1:2) crosslinker:actin concentration ratios, $r_{ABP:a}$. b, $\kappa_B$ for fascin-crosslinked bundles depends strongly on fascin concentration, $c_{fascin}$, in addition to bundle size ($r_{ABP:a}$ ● 1:2, ○ 1:10, ■ 1:20, □ 1:50). Deviations of the model from experiment at small bundle sizes ($n \leq 6$) are attributed to the disordered bundle structure likely present in that regime, which is not accounted for theoretically. c, The molecular length of $\alpha$-actinin is considerably longer than that of fascin or plastin (35 nm versus 10 nm) but has a shear stiffness similar to that of fascin (see text), $r_{ABP:a}$ ● 1:10, ○ 1:50. d, F-actin filaments bundled nonspecifically by PEG-induced depletion forces (4% PEG6k ●, 2% PEG6k ○) exhibit a $\kappa_B$ that increases quadratically in $n$ to large bundle sizes ($n \approx 30$), yielding a lower bound on the effective crosslinker stiffness per unit length of, $k_s / \delta \geq 10^4$ N/m². Dashed lines indicate decoupled ($\alpha \ll 1; \kappa_B = n k_\gamma$) and fully coupled ($\alpha \gg n; \kappa_B = n^2 \kappa_\gamma$) bundle bending regimes assuming zero inter-filament spacing ($t = 0$).
Figures

Figure 1
Figure 2
Figure 3

A

\[ \kappa_f (\mu \text{m}^2) \]
\[ \text{vs} \quad n \]

B

\[ \kappa_f (\mu \text{m}^2) \]
\[ \text{vs} \quad n \]

C

\[ \kappa_f (\mu \text{m}^2) \]
\[ \text{vs} \quad n \]

D

\[ \kappa_f (\mu \text{m}^2) \]
\[ \text{vs} \quad n \]