Synergy between Cyclase-associated protein and Cofilin accelerates actin filament depolymerization by two orders of magnitude

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Cellular actin networks can be rapidly disassembled and remodeled in a few seconds, yet in vitro actin filaments depolymerize slowly over minutes. The cellular mechanisms enabling actin to depolymerize this fast have so far remained obscure. Using microfluidics-assisted TIRF, we show that Cyclase-associated protein (CAP) and Cofilin synergize to processively depolymerize actin filament pointed ends at a rate 330-fold faster than spontaneous depolymerization. Single molecule imaging further reveals that hexameric CAP molecules interact with the pointed ends of Cofilin-decorated filaments for several seconds at a time, removing approximately 100 actin subunits per binding event. These findings establish a paradigm, in which a filament end-binding protein and a side-binding protein work in concert to control actin dynamics, and help explain how rapid actin network depolymerization is achieved in cells.
In control reactions, filaments depolymerized at their pointed ends at a rate of 0.14 ± 0.04 subunits s⁻¹ (±sd) whereas filaments incubated with 1 µM Cof1 depolymerized at 0.43 ± 0.1 subunits s⁻¹ (~3-fold faster; Fig. 1c, d, Supplementary Movie 1). Using fluorescently labeled Cy3-Cof1, we confirmed that this concentration of Cof1 rapidly decorates filaments along their lengths (Fig. 1d), which has been shown to prevent severing²⁶,²⁷. These effects of yeast Cof1 are similar to those reported for human Cof1-1 and ADF alone¹⁰,¹². In addition, 0.5 µM Srv2 alone increased pointed-end depolymerization by ~7-fold, to a rate of 0.96 ± 0.14 subunits s⁻¹, establishing Srv2 as an actin depolymerase.

Srv2 and Coflin synergize in depolymerizing pointed ends. When we combined Srv2 and Cofl, pointed-end depolymerization was accelerated by 330-fold to a rate of 43.9 ± 6.0 subunits s⁻¹ (Fig. 1c–e) (Supplementary Movie 1), approaching the estimated rates of actin turnover in vivo¹⁵. The rate observed in vitro for Cof1 and Srv2 together far exceeded the sum of the rates observed for each protein individually, indicating that these two proteins work synergistically to depolymerize actin. Put another way, this synergy of Cof1 with Srv2 leads to ~100-fold faster depolymerization than Cof1 alone. We then asked whether this in vitro activity can occur under physiological conditions where a high concentration of profilin-bound actin monomers is present (Fig. 1f). Adding 3 µM actin monomers (in presence or absence of 6 µM Profilin) did not appreciably alter synergistic depolymerization by Srv2 and Cofl, confirming that Srv2’s ability to interact with free actin monomers (through its C-terminus) does not hinder the depolymerization activities.

N-Srv2 mediates synergistic depolymerization with Cofl. Srv2/Cofl has two distinct functions in promoting actin turnover. Its hexamer-forming N-terminal half promotes filament turnover and its dimeric C-terminal half promotes monomer recycling. In addition, 0.5 µM Srv2 alone required that this concentration of profilin-1 and ADF alone10,12. In addition, 0.5 µM Srv2 alone increased pointed-end depolymerization by ~7-fold, to a rate of 0.96 ± 0.14 subunits s⁻¹, establishing Srv2 as an actin depolymerase.

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living cells dynamically rearrange their actin cytoskeletons in response to external signals in order to move, change shape, and reorganize their internal architecture¹. This remodeling requires rapid actin filament depolymerization. While this process occurs in seconds in vivo², purified actin filaments take minutes to depolymerize in vitro³–⁴. How accelerated depolymerization is achieved in vivo has been unclear. Coflin has long been recognized as a central player in promoting actin disassembly⁵–⁹. In addition to its ability to sever filaments, Coflin modestly accelerates depolymerization under physiological conditions, increasing the rate of subunit loss by about 4-fold (to ~1 subunit s⁻¹) at filament pointed ends¹⁰–¹². Twinfilin, another member of the actin depolymerization factor homology (ADF-H) family, also enhances depolymerization at filament ends. Yeast and mammalian Twinfilins alone can accelerate depolymerization at barbed ends, and in the presence of Cyclase-associated protein (CAP), yeast Twinfilin accelerates pointed-end depolymerization by ~20 fold, whereas mammalian Twinfilin does not¹³,¹⁴. These observations have established that ADF-H proteins can catalyze shortening at filament ends, but do not account for the estimated >100-fold faster actin depolymerization in vivo¹⁵. Thus, cellular mechanisms driving rapid pointed-end depolymerization are only partially understood.

CAP, also called Srv2 in Saccharomyces cerevisiae, is a conserved multidomain actin-binding protein found in animals, plants, and fungi¹⁶. Genetic and biochemical studies have linked Srv2/CAP to Coflin in controlling actin dynamics and actin-based cellular processes¹⁷–²¹. The N-terminal half of Srv2/CAP (N-Srv2/N-CAP) self-associates to form hexameric shuriken-like (bladed) structures that bind to the sides of actin filaments and enhance Coflin-induced severing by 4–8 fold²²,²³. The C-terminal half of Srv2/CAP (C-Srv2/C-CAP) dimerizes and binds with high affinity to Coflin-bound ADP-actin monomers, catalyzing the dissociation of Coflin and promoting nucleotide exchange on actin monomers¹⁷,¹⁹–²¹. Functions of N-Srv2/N-CAP in driving actin filament turnover in vitro and in vivo depend critically on a conserved actin-binding surface on its helical-folded domain (HFD)¹⁸,²³. This surface is also critical for the synergy between N-Srv2 and yeast Twinfilin in promoting actin depolymerization¹³. Thus, N-Srv2/N-CAP interactions with actin filaments are crucial for its known in vitro and in vivo functions.

Here we show that Srv2/CAP synergizes with Coflin to enhance pointed-end depolymerization by >300-fold, reaching speeds of up to ~50 subunits s⁻¹. Using microfluidics-assisted total internal reflection fluorescence (mf-TIRF) microscopy²⁴ and single-molecule imaging, we show that individual CAP hexamers interact transiently (on average for 2.2 ± 0.2 s) with the pointed ends of Coflin-decorated actin filaments and remove about 100 subunits each time.

**Results**

Srv2 is an actin filament depolymerase. Using mf-TIRF, we investigated the effects of S. cerevisiae CAP (Srv2) and Coflin (Cofl), individually and combined, at filament pointed ends (Fig. 1a, b). In these experiments, preformed fluorescently labeled actin filaments were flowed into a microfluidic chamber and captured at their barbed ends by capping protein (CapZ) anchored on the glass coverslip. Actin disassembly occurs more readily after subunits undergo ATP hydrolysis and phosphate release²⁵. Therefore, we incubated filaments for 15 min to allow Pᵢ release, and then exposed them to Cofl and/or full-length Srv2 (note: Srv2/CAP concentrations shown always refer to the concentration of the hexamers rather than monomers).

In control reactions, filaments depolymerized at their pointed ends at a rate of 0.14 ± 0.04 subunits s⁻¹ (±sd) whereas filaments incubated with 1 µM Cof1 depolymerized at 0.43 ± 0.1 subunits s⁻¹ (~3-fold faster; Fig. 1c, d, Supplementary Movie 1). Using fluorescently labeled Cy3-Cof1, we confirmed that this concentration of Cof1 rapidly decorates filaments along their lengths (Fig. 1d), which has been shown to prevent severing²⁶,²⁷. These effects of yeast Cof1 are similar to those reported for human Cof1-1 and ADF alone¹⁰,¹². In addition, 0.5 µM Srv2 alone increased pointed-end depolymerization by ~7-fold, to a rate of 0.96 ± 0.14 subunits s⁻¹, establishing Srv2 as an actin depolymerase.

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N-Srv2 mediates synergistic depolymerization with Cofl. Srv2/Cofl has two distinct functions in promoting actin turnover. Its hexamer-forming N-terminal half promotes filament turnover and its dimeric C-terminal half promotes monomer recycling. In addition, 0.5 µM Srv2 alone increased pointed-end depolymerization by ~7-fold, to a rate of 0.96 ± 0.14 subunits s⁻¹, establishing Srv2 as an actin depolymerase.

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rates could be measured. This analysis revealed no appreciable difference in the rates of pointed-end depolymerization induced by Cof1, Twf1 and N-Srv2 versus Cof1 and N-Srv2 (Fig. 2d).

Further analysis showed that at a fixed concentration of Cof1 (1 µM), N-Srv2 accelerated depolymerization in a concentration-dependent manner (Fig. 2e, $K_M = 50 \pm 10$ nM; $k_{cat} = 53 \pm 8$ subunits s$^{-1}$). This effect saturated near 150 nM N-Srv2, still well below the total cellular concentration of Srv2 in *S. cerevisiae* (0.5 µM hexamers)$^{13}$. These kinetics suggest that at lower N-Srv2 concentrations, the rate-limiting step in depolymerization may be binding of N-Srv2 hexamers to Cofilin-saturated filaments. At higher N-Srv2 concentrations, a different step in the mechanism (such as release of actin subunits from filament ends) may become rate-limiting.

Reciprocally, varying Cof1 concentration over a tenfold range (0.1–1 µM) at a fixed concentration of 0.5 µM N-Srv2 did not significantly alter the depolymerization rate (Supplementary Fig. 2). At even lower Cof1 concentrations (0.05 µM), depolymerization was slightly diminished, possibly due to incomplete decoration of filaments by Cof1. Reducing Cof1 concentration below 0.05 µM led to extensive severing, which precluded reliable measurement of depolymerization rates.

Fig. 1 *S. cerevisiae* Srv2/CAP and Cofilin synergize to accelerate actin filament depolymerization. a Schematic representation of the experimental strategy. Preformed Alexa-488-labeled actin filaments were captured by coverslip-anchored biotinylated SNAP-CapZ. After 15 min, 1 µM Cof1 and/or 0.5 µM Srv2 (or control buffer) was introduced into the chamber, and depolymerization was monitored. BE barbed end, PE pointed end. b Representative field of view showing anchored filaments aligned under flow, and the methodology used for determining the rate of pointed-end depolymerization from the slope of kymographs. Scale bar, 10 µm. c Rates (±sd) of pointed-end depolymerization in the presence of 1 µM Cof1 and/or 0.5 µM Srv2. Right: Magnified view of Control, Cof1, and Srv2 data. *statistical comparison by two-sample t test against Control (p < 0.05). Number of filament ends analyzed for each condition (left to right): 149, 55, 110 and 37. d Merged two-color kymograph of an Alexa-488-labeled actin filament (green), with 1 µM Cy3-Cof1 (red) introduced at the beginning of the red bar (see Supplementary Movie 1). e Same as (d) but with 1 µM Cy3-Cof1 (red) and 0.5 µM Srv2 (unlabeled). f Rates (±sd) of pointed-end depolymerization by 1 µM Cof1 and/or 0.5 µM Srv2 in the presence of 3 µM G-actin (with or without 6 µM Profilin). *statistical comparison by two-sample t test against Cof1 + Srv2 (p < 0.05). ns no evidence for significance at p = 0.05. Number of filament ends analyzed for each condition (left to right): 64, 83 and 84. Source data are provided as a Source Data file. All experiments were performed at least three independent times, and yielded similar results. Data shown are from one experiment.
Srv2/CAP and Cofilin synergy is evolutionarily conserved. Srv2/CAP homologs show remarkable conservation across plants, animals, and fungi in their domains, structure, and interactions. To address whether the synergistic depolymerization activity is conserved, we examined the activities of mammalian N-CAP1 in conjunction with mammalian Cofilins (Cofilin-1 or ADF). Similar to their yeast counterparts, mammalian N-CAP1, Cofilin-1, and ADF, each by itself had relatively modest depolymerization
effects, whereas N-CAP1 synergized with Cofilin-1 or ADF to accelerate depolymerization by ~250-fold and ~150-fold, respectively (Fig. 3a). These results indicate that the CAP-Cofilin synergy has been conserved across a billion years of evolution, from S. cerevisiae to mammals, and that it applies to both widely expressed isoforms of mammalian Cofilin.

Srv2/CAP and Cofilin synergy persists with phosphate present.

Actin filaments age via rapid ATP hydrolysis followed by slow Pi release (0.002 s−1). The latter step has long been considered to be rate-limiting in filament disassembly. Further, Cofilin preferentially binds to ADP-actin over ADP + Pi-actin, and sub-saturating concentrations of Cofilin exhibit drastically reduced severing activity in the presence of 20 mM free Pi (included to maintain filaments in the ADP + Pi state). All of our experiments presented so far were performed using aged ADP-actin filaments (after Pi release). To determine whether Srv2 and Cofilin synergy, like Cofilin alone, is sensitive to the nucleotide state of actin, we next included 20 mM Pi in the depolymerization reactions (Supplementary Table). These results indicate that the CAP-Cofilin synergy has been conserved across a billion years of evolution, from S. cerevisiae to mammals, and that it applies to both widely expressed isoforms of mammalian Cofilin.

Srv2/CAP hexamers transiently interact with pointed ends. To directly observe Cy5-Srv2/ΔCARP molecules on Cofilin−decorated filaments during depolymerization, we simultaneously imaged Alexa-488 actin and Cy5-Srv2/ΔCARP (83 nM) at high time resolution (0.065 s per frame) (Supplementary Movie 2). Cy5-Srv2/ΔCARP molecules preferentially bound to the pointed ends rather than the sides of filaments (Supplementary Fig. 5). Interactions at the pointed ends were transient, with an average dwell time of 2.2 ± 0.2 s (Fig. 4b−d), translating to a Cy5-Srv2/ΔCARP dissociation rate constant koff = 0.45 s−1. Further, we observed the shape of the intensity distribution of the pointed end-associated Cy5-Srv2/ΔCARP fluorescent spots (Supplementary Fig. 7) agreed with that predicted from the step photobleaching of surface-immobilized individual Cy5-Srv2/ΔCARP molecules (Supplementary Fig. 5), suggesting that pointed end-bound molecules are single hexamers.

We also determined the kinetics of Srv2/ΔCARP association with the filament pointed end by measuring the average length of time from the end of one Cy5-Srv2/ΔCARP binding event to the beginning of the next, and then correcting (see Methods) for the fraction of molecules that are unlabeled. This measurement yielded a second-order association rate constant kass = 1.1 ± 0.2 × 107 s−1 M−1. This extremely fast rate approaches the expected diffusion-limited rate constant for a large molecule the size of a Srv2 hexamer associated with a filament end. The single-molecule observations also provide insights into the mechanism of synergistic depolymerization. At 83 nM Cy5-Srv2/ΔCARP, the filament end is occupied by Srv2 only ~15% of the time, and filaments depolymerize at only ~15% of the maximal rate seen at saturating Srv2 concentrations (Table 1). Similarly, at 83 nM Cy5-Srv2/ΔCARP, the filament end is occupied ~65% of the time, and filaments depolymerize at ~65% of the maximal rate. This parallel between end occupancy and depolymerization rate suggests a model in which the pointed end depolymerizes rapidly when Srv2 is bound to the filament end. Consistent with the model, at the lower (83 nM) concentration of Srv2, we observe two distinct behaviors: brief periods of rapid depolymerization interspersed with longer periods of little or no depolymerization (Fig. 4e, red). The two behaviors are less obvious at 83 nM, likely because the individual Srv2 binding events are too closely spaced in time (e.g., Fig. 4c) to be well resolved in the velocity data (Fig. 4e, black). Overall, this
model predicts that the filament end is almost continuously occupied at saturating concentrations of Srv2 (>~150 nM; Fig. 2e), because as soon as one Srv2 hexamer dissociates, it is almost immediately replaced by another. However, at Srv2 concentrations well below $K_M$ (Fig. 2e), the pointed end is occupied only a small fraction of the time, giving rise to slower rates of depolymerization.

To test the model, we examined filament depolymerization during individual Srv2 binding events. This analysis is difficult because the precision of actin filament length measurement is limited in our experiments by filament Brownian motion and partial (10%) fluorescent labeling of actin monomers. Nevertheless, it is clear from examples of unusually long Srv2 binding events that while some of these events are accompanied by rapid
depolymerization (e.g., Fig. 4f), some are not (e.g., Fig. 4g). As expected, a histogram of all depolymerization velocities measured at 8.3 nM Cy5-Srv2ΔCARP when the protein was absent from the filament end showed a narrow peak centered close to zero (Fig. 4h, top). In contrast, velocities when Cy5-Srv2ΔCARP was present at the end showed a broader distribution, including large velocities (consistent with rapid depolymerization) as well as a significant number of velocities close to zero. Thus, the data in Fig. 4f–h suggest that not all single-molecule Srv2 binding events at the filament pointed end produce rapid depolymerization. It is possible that this heterogeneity is caused by differences in the nature of Srv2 hexamer interactions with the filament end and/or how well the end of the filament is decorated by Cof1.

Despite the fact that we could not accurately measure depolymerization velocities during Srv2 binding events shorter than those selected for Fig. 4g, h, we could still calculate how many actin subunits were removed from the pointed end on average during each Srv2 binding event. From the saturation kinetics of pointed-end depolymerization, we calculated $k_{\text{cat}}/K_{M} = 106 \pm 27 \times 10^{7}$ actin subunits $s^{-1} M^{-1}$ (Fig. 2e), which is the mean number of actin subunits removed per binding event multiplied by the association rate constant31. The latter quantity, $k_{\text{on}} = 1.1 \pm 0.2 \times 10^{7} s^{-1} M^{-1}$, was measured in the single-molecule experiments described above. The ratio of these values yields the mean number of subunits removed per Srv2ΔCARP binding event, $96 \pm 30$ subunits. Thus, on average each binding of an individual ~40 nm diameter Srv2 hexamer20 reduces the filament length by ~270 nm.

Discussion

Cellular actin filament networks must be dynamically assembled and turned over, and their monomeric actin building blocks rapidly recycled for new rounds of polymerization. Two members of the ADF-homology superfamily, Cofilin and Twinfilin, have been implicated in promoting actin depolymerization8,10,12–14. Alone, Twinfilin processively tracks the barbed ends of filaments and modestly enhances their rate of depolymerization. Cofilin both severs filaments and modestly enhances the depolymerization rate at filament ends. Neither protein alone can enhance pointed-end depolymerization to rates of depolymerization expected to occur in vivo.

In this study, we have elucidated a multicomponent mechanism in which Cofilin and Srv2/CAP, which individually have only modest depolymerization effects, together produce a dramatic >300-fold acceleration of depolymerization at filament pointed ends. We show that Srv2/CAP hexamers transiently bind to the pointed end of Cofilin-decorated filaments, with each binding event on average leading to the removal of about 100 actin subunits. It is unlikely that this number of actin subunits leave in a complex with a single departing Srv2 hexamer. Instead, we propose that the subunits dissociate as free actin monomers, Cofilin-bound actin monomers, and/or very short oligomers (Fig. 5a).

Together with previous observations, our results demonstrate that Srv2/CAP can affect actin dynamics in four distinct ways. First, as we show here, and in agreement with earlier bulk studies17, Srv2/CAP alone enhances pointed-end depolymerization. Our direct observation of these effects on individual actin filaments by TIRF microscopy establish Srv2/CAP as a bona fide pointed-end depolymerase. Further, our results reveal that in conjunction with filament decoration by Cofilin, these interactions of Srv2/CAP promote extraordinarily fast rates of pointed-

![Fig. 5 Working model for Srv2/CAP, Cofilin, and Twinfilin functions in actin disassembly. a Srv2 hexamers bind transiently (for ~2 s on average) to the pointed ends of Cofilin-saturated actin filaments. In a subset of these binding events, Srv2 catalyzes the dissociation of actin subunits. Subunits might be released as actin monomers, Cofilin-bound monomers, and/or Cofilin-bound oligomers. b On filaments that are more sparsely decorated with Cofilin, Srv2/CAP can bind to filament sides and enhance Cofilin-mediated severing, in addition to accelerating pointed-end depolymerization of the severing products. Twinfilin interacts with filament barbed ends to promote their depolymerization, where its interactions with Srv2/CAP increase its processivity. The thickness of arrows indicates relative rates of polymerization or depolymerization at the two ends of the filament.](image-url)
end depolymerization. Second, the C-terminal half of Srv2/CAP catalyzes displacement of Cofflin from ADP-actin monomers and promotes nucleotide exchange, possibly in collaboration with its binding partner Profilin17,19,21,32,33. Our results here show that the presence of actin monomers (with or without Profilin) does not alter Srv2/CAP synergy with Cofflin in pointed-end depolymerization, demonstrating the potential for this depolymerization activity to occur under physiological conditions in vivo, where micromolar concentrations of profilin-bound actin monomers are present. Third, the N-terminal half of Srv2 increases the progressivity of Twinfilin at barbed ends, resulting in longer depolymerization runs induced by Twinfilin14. Fourth, N-Srv2 also enhances Cofflin-mediated severing of filaements, by directly interacting with filament sides18,22. This occurs at lower levels of Cofflin, where filament sides are sparsely decorated30. In the present study, we have shown that at higher concentrations of Cofflin, as found in vivo, Cofflin more completely decorates filament sides, and Srv2 no longer enhances severing but promotes rapid pointed-end depolymerization. Depolymerization effects are mediated by N-Srv2, and require direct interactions with Cofflin filaments, and are dependent on a conserved surface on the HFD domain18,22,23. An accompanying manuscript makes similar observations and provides the structural basis for N-Srv2/N-CAP interactions with the pointed ends of filaments34.

How might the two filament-disassembly activities of Srv2/CAP (enhancement of severing and synergistic depolymerization) contribute to actin turnover in vivo? In the complex cellular milieu, there are many actin filament side-binding proteins that compete with Cofflin, producing discontinuities in Cofflin decoration to promote severing26,27. When this occurs, Srv2 is likely to bind filament sides and enhance severing, in addition to interacting with pointed ends of filaments (including Cofflin-decorated products of severing) and catalyze depolymerization (Fig. 5b). How Twinfilin-mediated depolymerization contributes to cellular actin disassembly is not well understood, but our data suggest that Twinfilin can enhance Cofflin-mediated severing by interrupting Cofflin decoration on filament sides. In addition, Twinfilin binds with high affinity to Capping Protein, and Twinfilin functions have been linked genetically and biochemically to Capping Protein36–38. Therefore, Twinfilin may have a particularly important role in controlling dynamics at barbed ends (Fig. 5b).

In summary, our results uncover a synergistic, conserved mechanism of actin depolymerization driven by Srv2/CAP and Cofflin. These results establish a paradigm in which an actin filament end-binding protein and a side-binding protein work in concert to govern actin dynamics. Further, they fill a major gap in our understanding of how actin networks can be disassembled rapidly, and may account for the high rates of depolymerization hypothesized to occur in vivo, e.g., at the leading edge and sites of endocytosis2,29.

Methods

**Purification and labeling of rabbit muscle actin.** Rabbit skeletal muscle actin was purified from acetone powder39 generated from frozen ground hind leg muscle tissue of young rabbits (PelFreez, Rogers, AR). Lyophilized acetone powder stored at −80 °C was mechanically sheared in a coffee grinder, resuspended in G-buffer (5 mM Tris·HCl pH 7.5, 0.5 mM Dithiothreitol (DTT), 0.2 mM ATP, 0.1 mM CaCl2), and then cleared by centrifugation for 20 min at 50,000 × g. Actin was polymerized by the addition of 2 mM MgCl2 and 50 mM NaCl and incubated overnight at 4 °C. F-actin was pelleted by centrifugation for 150 min at 361,000 × g at 4 °C. The supernatant was then incubated with glutathione-agarose beads for 1 h at room temperature. The pellet was resuspended in lysis buffer (20 mM NaPO4 pH 7.8, 300 mM NaCl, 1 mM DTT, 15 mM imidazole), eluted protein was concentrated and labeled with Benzylguanine-Biotin (New England Biolabs) according to the manufacturer’s instructions. Free biotin was removed using size-exclusion chromatography by loading the labeled protein on a Superose 6 gel-filtration column (GE Healthcare, Pittsburgh, PA) eluted with 20 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM DTT. Fractions containing the protein were combined and concentration was determined by measuring the absorbance (ε280 = 102,165 M−1 cm−1). Purified protein was aliquoted, snap frozen in liquid N2 and stored at −80 °C.

**Purification and labeling of ADF/Coflin.** Wild-type yeast Coflin (Cof1) was purified as follows42. The protein was expressed fused to a glutathione-S-transferase (GST) tag with a thrombin cleavage site and expressed in E. coli BL21 DE3. Cells were grown to log phase at 37 °C in TB medium, then induced with 1 mM IPTG at 18 °C overnight. Cells were harvested by centrifugation and pelleted were stored at −80 °C. Frozen pellets were resuspended in lysis buffer (20 mM NaPO4 pH 7.8, 300 mM NaCl, 1 mM DTT and 15 mM imidazole) to remove nonspecifically bound proteins. SNAP-Cof1 was then eluted with a linear zero to 250 mM imidazole gradient in 20 mM NaPO4 pH7.8, 300 mM NaCl, and 1 mM DTT. The eluted protein was concentrated and labeled with Benzylguanine-Biotin (New England Biolabs) according to the manufacturer’s instructions. Free biotin was removed using size-exclusion chromatography by loading the labeled protein on a Superose 6 gel-filtration column (GE Healthcare, Pittsburgh, PA) eluted with 20 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM DTT. Fractions containing the protein were combined and concentration was determined by measuring the absorbance (ε280 = 102,165 M−1 cm−1). Purified protein was aliquoted, snap frozen in liquid N2 and stored at −80 °C.

**Purification and biotinylation of SNAP tagged CapZ.** SNAP-CapZ41 was expressed in E. coli BL21 DE3 (Stratagene, La Jolla, CA) by growing cells to log phase at 37 °C in TB medium, then inducing expression using 1 mM IPTG at 18 °C overnight. Cells were harvested by centrifugation and pellets were stored at −80 °C. Frozen pellets were resuspended in lysis buffer (20 mM NaPO4 pH 7.8, 300 mM NaCl, 1 mM DTT, 15 mM imidazole) to remove nonspecifically bound proteins. SNAP-CapZ was then eluted with a linear zero to 250 mM imidazole gradient in 20 mM NaPO4 pH7.8, 300 mM NaCl, and 1 mM DTT. The eluted protein was concentrated and labeled with SNAP-CapZ was then eluted with a linear zero to 250 mM imidazole gradient in 20 mM NaPO4 pH7.8, 300 mM NaCl, and 1 mM DTT. The eluted protein was concentrated and labeled with Benzylguanine-Biotin (New England Biolabs) according to the manufacturer’s instructions. Free biotin was removed using size-exclusion chromatography by loading the labeled protein on a Superose 6 gel-filtration column (GE Healthcare, Pittsburgh, PA) eluted with 20 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM DTT. Fractions containing the protein were combined and concentration was determined by measuring the absorbance (ε280 = 102,165 M−1 cm−1). Purified protein was aliquoted, snap frozen in liquid N2 and stored at −80 °C.

**Purification and biotinylation of Twinfilin.** Twinfilin was purified as follows42. The protein was expressed fused to a glutathione-S-transferase (GST) tag with a thrombin cleavage site and expressed in E. coli BL21 DE3. Cells were grown to log phase at 37 °C in TB medium, then induced with 1 mM IPTG at 18 °C overnight. Cells were harvested by centrifugation and pellets were stored at −80 °C. Frozen pellets were resuspended in lysis buffer (20 mM NaPO4 pH 7.5, 150 mM NaCl and 1 mM DTT) to remove unbound protein and then incubated with thrombin (0.05 mg/ml) to cleave Coflin from bead-bound GST. The cleaved protein was recovered by centrifugation. The supernatant containing the protein was concentrated and loaded on to a Superose 12 gel-filtration column (GE Healthcare, Pittsburgh, PA) equilibrated with 10 mM Tris·HCl pH 7.5, 50 mM NaCl and 0.1 mM DTT. The fractions containing Coflin were pooled, concentrated, snap frozen in liquid N2 and stored at −80 °C. To prepare fluorescently labeled Coflin, Cof1(T46C/C62A)18 was purified as described above and dialyzed overnight against 10 mM Tris·HCl pH 7.5, 50 mM NaCl, and 0.2 mM Tris (2-carboxyethyl)phosphine (TCEP) at 4 °C. The dialyzed protein was then mixed with a 10-fold molar excess of Cy3-maleimide (GE Healthcare, Pittsburgh, PA) and incubated overnight in the dark at 4 °C. Free dye was removed using a PD-10 desalting column. The labelled protein was then aliquoted, snap frozen in liquid N2 and stored at −80 °C. Human Coflin and ADF were purified as follows43. The proteins were expressed in E. coli BL21 DE3 by growing cells to log phase at 37 °C in TB medium, then induced with 1 mM IPTG at 18 °C overnight. Cells were harvested by centrifugation and pellets were stored at −80 °C. Frozen pellets were resuspended in 20 mM Tris pH 8.0, 50 mM NaCl, 1 mM DTT, and protease inhibitors as described above. Cells were lysed by sonication with a tip sonicator while keeping the tubes on ice. The lysate was cleared by centrifugation at 150,000 × g for 30 min at 4 °C. The supernatant was then flowed through a HisTrap column connected to a Fast Protein Liquid Chromatography (FPLC) system. The column with the bound protein was extensively washed with the washing buffer (20 mM NaPO4 pH 7.8, 300 mM NaCl, 1 mM DTT and 15 mM imidazole) to remove nonspecifically bound proteins. SNAP-Cof1 was then eluted with a linear zero to 250 mM imidazole gradient in 20 mM NaPO4 pH7.8, 300 mM NaCl, and 1 mM DTT. The eluted protein was concentrated and labeled with Benzylguanine-Biotin (New England Biolabs) according to the manufacturer’s instructions. Free biotin was removed using size-exclusion chromatography by loading the labeled protein on a Superose 6 gel-filtration column (GE Healthcare, Pittsburgh, PA) eluted with 20 mM HEPES pH 7.5, 150 mM KCl, 0.5 mM DTT. Fractions containing the protein were combined and concentration was determined by measuring the absorbance (ε280 = 102,165 M−1 cm−1). Purified protein was aliquoted, snap frozen in liquid N2 and stored at −80 °C.
Healthcare, Pittsburgh, PA), and the flow-through was harvested and dialyzed against 20 mM HEPES pH 6.8, 25 mM NaCl, and 1 mM DTT. The dialyzed solution was then loaded on a 1 ml HiTrap SP FF column (GE Healthcare, Pittsburgh, PA) and eluted using a linear gradient of NaCl (20–500 mM). Fractions containing ADP/Coflin were concentrated, dialyzed into 20 mM Tris pH 8.0, 50 mM KCl, and 1 mM DTT, snap frozen in liquid N2 and stored at −80°C.

**Purification and labeling of Srv2/CAP polykettides.** His-tagged full-length *S. cerevisiae* Srv2, 2–90, N-terminal fragments N-Srv2, Srv2–CARP (Fig. 2a) and mouse N-CAP122 were expressed in *Escherichia coli* BL21 DE3 by growing cells to log phase at 37°C in TB medium. Cells were induced with 1 mM IPTG at 18°C overnight. Cells were harvested by centrifugation and pellets were stored at −80°C. Frozen pellets were resuspended in 50 mM NaPO₄ pH 8.0, 1 mM PMSF, 1 mM DTT, 20 mM imidazole, 300 mM NaCl and protease inhibitors as described above. Cells were lysed by sonication with a tip sonicator while keeping the tubes on ice to 60% lysis. The lysate was cleared by centrifugation at 150,000 × g for 30 min at 4°C. The lysate was loaded on a 1 ml HisTrap HP column (GE Healthcare, Pittsburgh, PA) and eluted using a linear gradient of NaCl (20–100 mM) for 30 min at 25°C and additionally for 14 h at 4°C. The excess dye was then quenched by addition of 5 mM DTT. Free dye was then separated from the bound dye by size-exclusion chromatography on a Superose 12 column (GE Healthcare) equilibrated in 20 mM HEPES pH 7.5, 1 mM EDTA, 50 mM KCl and 0.5 mM DTT. Peak fractions were pooled, aliquoted, snap frozen in liquid N2 and stored at −80°C. For fluorescent labeling of Srv2/CARP, the same procedure as above was followed with the addition that the elution buffer was replaced with 0.2 mM Tris (2-carboxyethyl)phosphine (TCEP) and the eluate was concentrated and dialyzed into 10 mM imidazole pH 8.0, 150 mM NaCl and 1 mM DTT. The protein was then aliquoted, snap frozen in liquid N2 and stored at −80°C. For labeling of Twinfilin, *S. cerevisiae* Twinfilin Twfl was expressed as a GST-fusion protein13 in *Escherichia coli* BL21 DE3 by growing cells to log phase at 37°C in TB medium. Cells were induced with 0.4 mM IPTG at 18°C overnight. Cells were harvested by centrifugation and pellets were stored at −80°C. Periodic acid was used to clean the coverslips, which were coated with a 80% ethanol solution in an N2 stream. The cleaned coverslips were coated with a 1% BSA and 0.5% Tween-20 solution in TIRF buffer. Each cell was then rinsed with TIRF buffer and incubated with 1% BSA and 10 μg/ml streptavidin in TIRF buffer for 5 min. Actin filaments were anchored along their lengths with the exception that 1 mM DTT in the elution buffer was replaced with 0.2 mM DTT. Peak fractions were pooled, aliquoted, snap frozen in liquid N2 and stored at −80°C.

**Microfluidics-assisted TIRF microscopy.** Actin filament depolymerization was monitored by microfluidics-assisted Total Internal Reflection Microscopy (mf-TIRF)12,24,46. Coverslips were first cleaned by sonication in detergent for 60 min. Cells were harvested by centrifugation and pellets were stored at −80°C. Cell pellets were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.2% Triton X-100, lysozyme + protease inhibitors as described above), kept on ice for 30 min, and then further lysed by sonication. Lysates were cleared for 25 min at 272,000 × g at 4°C, and the supernatant was collected and loaded on a HiTrap Q column (Buffer: 20 mM Tris-HCl pH 8.0) followed by a Superdex 200 column equilibrated in 20 mM HEPES pH 8.0, 50 mM NaCl. Peak fractions were pooled, snap frozen in aliquots, and stored at −80°C.

**Data availability**

Data supporting the findings of this manuscript are available from the corresponding authors upon reasonable request. A reporting summary for this article is available as a Supplementary Information file. The source data underlying Figs. 1c, 1f, 2b, 3a, b, 4d, 4h are provided as a Data Source file.

**Code availability**

Custom-written LabVIEW software (GLIMPSE) used for image acquisition in single-molecule experiments can be accessed at https://github.com/gelles-brandeis/Glimpse.
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Author contributions
S.S., J.G. and B.L.G. designed the experiments and wrote the manuscript, S.S. and J.C. performed experiments, and S.S., J.C., and J.K. analyzed the data.

Competing interests
The authors declare no competing interests.

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