

A novel mechanism of actin filament processive capping by formin: solution of the rotation paradox

Tom Shemesh,¹ Takanori Otomo,² Michael K. Rosen,² Alexander D. Bershadsky,³ and Michael M. Kozlov¹

¹Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel

²Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390

³Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

The FH2 domains of formin family proteins act as processive cappers of actin filaments. Previously suggested stair-stepping mechanisms of processive capping imply that a formin cap rotates persistently in one direction with respect to the filament. This challenges the formin-mediated mechanism of intracellular cable formation. We suggest a novel scenario of processive capping that is driven by developing and relaxing torsion elastic stresses. Based on the recently discovered crystal structure of an FH2–actin complex, we propose a second

mode of processive capping—the screw mode. Within the screw mode, the formin dimer rotates with respect to the actin filament in the direction opposite to that generated by the stair-stepping mode so that a combination of the two modes prevents persistent torsion strain accumulation. We determine an optimal regime of processive capping, whose essence is a periodic switch between the stair-stepping and screw modes. In this regime, elastic energy does not exceed feasible values, and supercoiling of actin filaments is prevented.

Introduction

Formin family proteins nucleate actin polymerization and remain bound to the barbed ends of actin filaments, enabling filament growth in the barbed direction at the same time (for review see Higgs, 2005). The latter phenomenon, which is referred to as processive or “leaky” capping, has been directly visualized in cells (Higashida et al., 2004) and in vitro systems (Kovar and Pollard, 2004; Romero et al., 2004). Formin-driven actin polymerization and filament growth are involved in multiple intracellular processes such as formation of the linear actin bundles, cell movement, and cytokinesis (for review see Wallar and Alberts, 2003; Zigmond et al., 2003; Pollard, 2004; Watanabe and Higashida, 2004).

A minimal protein module that is necessary for processive capping is a dimer of the formin homology domain FH2 (for review see Higgs, 2005). While attached to the filament barbed end, an FH2 dimer allows for barbed end polymerization at rates equal to or lower than that of a pure actin filament (Zigmond et al., 2003). The acceleration of processive capping polymerization requires a complex of the formin homology domain FH1 with profilin in addition to FH2 dimer (Romero et al., 2004).

Crystallographic, nuclear magnetic resonance, and biochemical data indicate that the FH2 domain is dimeric in its functional form (Li and Higgs, 2003; Xu et al., 2004; Otomo et

al., 2005). This dimer is composed of two structural units that are termed actin bridge elements and are reciprocally connected by flexible tethers to form a topologically closed ring. In the Bni1p–FH2 domain complex with tetramethylrhodamine-actin, each bridge binds to two actin monomers in an orientation that closely resembles a short-pitch actin dimer. This suggests that this structure could be a nucleus from which a new filament could grow (Otomo et al., 2005). On the basis of this structure, it was proposed that the FH2 dimer at the barbed end can exist in two configurations (termed closed and open) that differ in the relative position and orientation of the two bridges (Otomo et al., 2005). In the closed configuration, which is blocked for the addition of new actin monomers, bridges bind the three terminal actins in such a manner that the first bridge binds the protruding (actin 1) and penultimate (actin 2) actin subunits, whereas the second bridge binds the penultimate (actin 2) and the following (actin 3) subunits (Fig. 1 a). In the open configuration, which is competent for the monomer addition, the two bridges bind only the two terminal actins; one bridge is bound to actins 1 and 2, whereas the second bridge binds only actin 1 and exposes its post domain to bind a new actin monomer (Fig. 1, b and c).

The essence of actin polymerization upon processive capping is a repeating transition of the barbed end–formin complex from the closed to the open state followed by the addition of a new actin monomer to the filament. It has been proposed that the rate of this transition is modulated by elastic stresses, which accumulate within the formin–actin complex in the closed state

Correspondence to Michael M. Kozlov: michk@post.tau.ac.il

Abbreviation used in this paper: NEM, N-ethylmaleimide.

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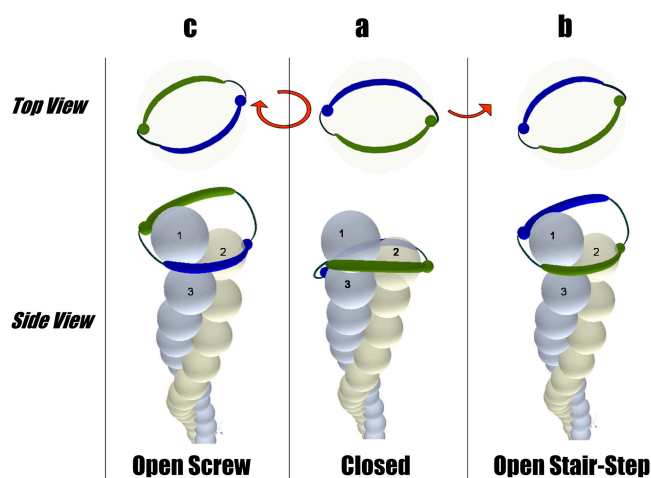


Figure 1. Two modes of processive capping of actin filaments by a dimer of formin homology domain FH2. The model is based on the structure of an FH2–formin complex that was established crystallographically (Otomo et al., 2005). Spheres represent the actin monomers. The formin bridges are shown as blue and green elongated bodies winding around the actin filament. Red arrows indicate the directions of FH2 rotation with respect to the filament bulk. (a) The closed state of the formin–actin complex, which is unavailable for insertion of new actin monomers. The green bridge binds the protruding (actin 1) and penultimate (actin 2) subunits, whereas the second, blue bridge binds actins 2 and 3 subunits. (b) The stair-stepping mode of processive capping. The blue bridge migrates from actins 2 and 3 to actin 1 and exposes its post domain for insertion of a new actin monomer. The FH2 dimer rotates by $\sim 14^\circ$ in the direction of twist of the long-pitch actin helix. (c) The screw mode of processive capping. The two bridges undergo a screwlike motion around the filament until they bind in the new position corresponding to the open state of the filament end. The FH2 dimer rotates in the direction of the short-pitch actin helix, which is opposite to the rotation direction of the stair-stepping mode. Rotation angle in the screw mode is approximately -166° .

(Kozlov and Bershadsky, 2004; Otomo et al., 2005). The origin of elastic stresses may lie in deformation of the barbed end helical structure by FH2 bridges (Otomo et al., 2005), deformation of the FH2 dimer itself (Kozlov and Bershadsky, 2004), or a combination of the two kinds of deformations (Kozlov and Bershadsky, 2004). A transition from the closed to the open state results in the relaxation of these stresses, whereas the insertion of new actin monomer again reloads the “formin–actin spring,” preparing it for a new cycle of processive capping (Kozlov and Bershadsky, 2004; Otomo et al., 2005). The detailed mode of this transition, as has been proposed previously (Otomo et al., 2005), involves the migration of one FH2 bridge from actin subunits 2 and 3 to actin 1 through movement along the filament axis, whereas the second bridge remains bound to actins 1 and 2 (Fig. 1 b). At the next polymerization step (after the insertion of a new actin monomer), the second bridge migrates to the terminal actin. Because of the steplike movement of the FH2 dimer within this scenario, we refer to it as the stair-stepping mode, which is a term that was introduced by Xu et al. (2004).

Although the stair-stepping mode explained many aspects of FH2 function and is consistent with the major phenomenology of processive capping, it made one counterintuitive prediction (Pollard, 2004). Because of the helical structure of the actin filament, stair stepping requires FH2 to rotate relative to the filament. Each stair-stepping step is coupled to rotation

of the formin dimer with respect to the bulk of the filament in the direction of twist of long-pitch actin helices (Fig. 1 b). The rotation angle constitutes $\sim 14^\circ$, which is half of the angle between the sequential actin monomers in a long-pitch actin helix. Such rotation is difficult to reconcile with the assembly of cross-linked bundles of actin filaments (Pollard, 2004) in budding yeast (Yang and Pon, 2002) and from adherens junctions (Kobiela et al., 2004). A continuous turning of filament ends with respect to filament bodies, which are interconnected within the cables, would generate a persistent accumulation of elastic torsion strains and stresses in the system. This would be incompatible with continuous polymerization and generate filament supercoiling. Attempts to observe a turning of the bulks of polymerizing actin filaments with respect to their formin caps have been undertaken in vitro in one-filament experiments in which the filament barbed and pointed ends were firmly attached to the substrate via the Bni1–FH2 cap and an inactivated myosin tether, respectively (Kovar and Pollard, 2004). In these experiments, tight binding between the barbed end and FH2 cap was confirmed by $>10\text{-}\mu\text{m}$ filament elongation, which was accompanied by considerable buckling before filament release from the FH2 cap (Kovar and Pollard, 2004; for review see Higgs, 2005). The experiments revealed neither persistent filament rotation with respect to the substrate nor filament supercoiling. An alternative to filament rotation could be a persistent turning of the FH2 cap with respect to the substrate, but this may not be a general solution to the problem. Coupling between FH2 dimer turning and processive capping represents the “rotation paradox” of the stair-stepping model (Kovar and Pollard, 2004; Pollard, 2004).

Results and discussion

Based on the crystal structure of the formin–barbed end complex (Otomo et al., 2005), we propose a second mode of transition from the closed to the open state, which we will refer to as the screw mode. This mode involves screwlike movement of the FH2 dimer around the barbed end, as illustrated in Fig. 1 c. Within the screw mode, each FH2 bridge moves along the short-pitch actin helix. As a result, one bridge undergoes a transition from actins 1 and 2 to actin 1 and opens its post site for binding of a new actin monomer, whereas the second bridge moves from actins 2 and 3 to actins 1 and 2 (Fig. 1 c). The twist direction of the short-pitch actin helix is opposite to that of the long-pitch helix (Holmes et al., 1990). Therefore, rotation of the FH2 dimer in the screw mode is opposite to that of the stair-stepping mode. Transition from the closed to the open state within the screw mode is coupled to rotation of the FH2 dimer with respect to the bulk of the filament by $\sim 166^\circ$, which is equal to the angle between two sequential monomers in the short-pitch actin helix (Fig. 1 c).

For the following treatment, we refer to rotation of the formin dimer with respect to the filament bulk as torsion and define it as positive for the stair-stepping mode and as negative for the screw mode. According to this definition, the torsion angle of one stair-stepping step is $\phi_{SS} \approx 14^\circ$, whereas that of the screw step is $\phi_{SCR} \approx -166^\circ$. In the case that is relevant for

this study, the formin cap is fixed on a membrane or substrate and cannot freely rotate as required by the helical structure of the filament. In this case, the rotation of formin relative to the filament bulk is substituted by the torsional deformation of the actin filament (see supplemental material, available at <http://www.jcb.org/cgi/content/full/jcb.200504156/DC1>). This deformation will be referred to as the torsion strain (τ).

The most prominent difference between the two modes is in the opposite directions of FH2 dimer rotation with respect to the actin filament bulk. We propose that, as a result of this feature, torsion strains that are produced by the two modes can mutually compensate, and, hence, processive capping consisting of an optimal combination of the two modes is largely free of the rotation paradox. Indeed, a straightforward consideration shows that torsion strain produced by a sequence of ~ 12 stair-stepping steps that require the relative actin filament–formin rotation by $\sim 14^\circ$ each can be compensated for by one screw step that generates rotation in the opposite direction by approximately -166° . Hence, processive capping that consists of repeated sequences of a mean of ~ 12 stair-stepping steps for each screw step will not lead to a persistent one-directional formin rotation and to the related problems of stress–strain accumulation within growing actin filaments. Stress–strain relaxation by periodic switching between the stair-stepping and screw modes will prevent filament supercoiling. Thus, a combination of the stair-stepping and screw modes could enable an essentially torsion stress–free polymerization of long actin cables in vivo (Sagot et al., 2002; Yang and Pon, 2002) and could explain the absence of relative rotation of the formin cap and actin filament, as was observed in vitro (Kovar and Pollard, 2004).

To substantiate these conclusions, we performed an elastic energy analysis of the process of actin filament polymerization upon processive capping by a formin dimer (see supplemental material for mathematical details and a more expanded analysis). We considered an elastic model of a system that consists of an actin filament undergoing polymerization upon processive capping by a formin dimer. We assumed that at each step of polymerization, the system can choose between two alternative modes (the stair-stepping and screw mode), contributing 14 and -166° , respectively, to the torsion strain that accumulated as a result of previous steps. The essence of the analysis was to calculate and compare the energies of the two alternatives. Using a deterministic approach, we stated that the mode of the polymerization step undertaken by the system is that of the smaller energy. By performing this analysis step by step, we determined the optimal regime of polymerization and accompanying variations in the torsion strain and elastic energy. A more general probabilistic treatment of processive capping steps is expected to give qualitatively similar results and will be published elsewhere.

To be specific and make quantitative estimations, we used parameters corresponding to the experimental design of Kovar and Pollard (2004). The system consists of a growing actin filament, whose barbed end is capped by a formin molecule that is attached to a substrate. When the filament reaches a length of $\sim 1 \mu\text{m}$, its pointed end is also fastened to the substrate through

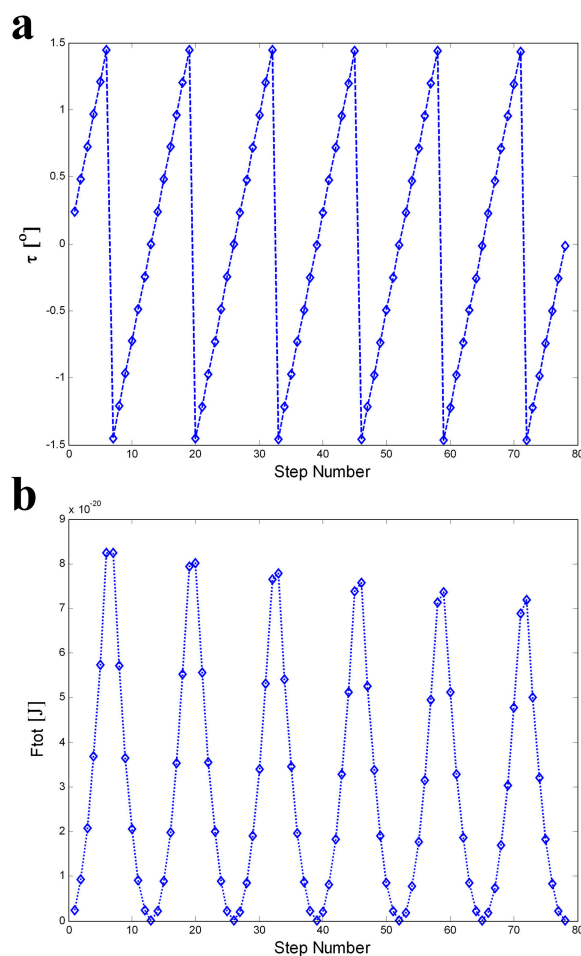


Figure 2. The optimal regime of processive capping, consisting of repeated sequences of ~ 12 stair-stepping steps followed by one screw step. (a) The torsion strain as a function of the number of polymerization steps after the beginning of elastic stress accumulation. The torsion strain changes periodically between the values of approximately -83° and 83° . Regions of the positive slope correspond to the stair-stepping mode, whereas regions of the negative slope represent the screw mode. (b) Change of elastic energy (F_{tot}) in the course of stair stepping. The elastic parameters used in the calculation are the actin filament torsion modulus $C \approx 8 \times 10^{-26} \text{ J} \times \text{m}$ (Tsuda et al., 1996) and bending modulus $K \approx 3.6 \times 10^{-26} \text{ J} \times \text{m}$ (Gittes et al., 1993; Isambert et al., 1995). It is also assumed that the torsion energy starts to accumulate after the filament reaches a length of $1 \mu\text{m}$, which corresponds to the experimental design of Kovar and Pollard (2004). The energy changes periodically with slowly decreasing amplitude. Maximal energy is reached in the first cycle and does not exceed $\sim 20 k_B T$, where $k_B T \approx 0.6 \text{ kcal/M}$ is the product of the Boltzmann constant and absolute temperature.

an *N*-ethylmaleimide (NEM)–treated myosin II molecule. After the pointed end is immobilized, free rotation of the filament is prevented, and further polymerization gives rise to torsion stresses within the system. In general, these stresses are shared between the actin filament on one hand and formin and NEM–myosin on the other. To estimate the maximum possible torsion stresses, we assumed formin, NEM–myosin, and their links to the substrate to be much more rigid than the actin filament, whose rigidity is characterized by two elastic moduli: the torsion modulus $C \approx 8 \times 10^{-26} \text{ J} \times \text{m}$ (Tsuda et al., 1996) and the bending modulus $K \approx 3.6 \times 10^{-26} \text{ J} \times \text{m}$ (Gittes et al., 1993; Isambert et al., 1995). A general case of stress distribution is considered in

the supplemental material. Although performed for a particular experimental design, the results of the analysis also apply qualitatively to *in vivo* actin–formin systems such as intracellular actin cables (Yang and Pon, 2002; Kobiela et al., 2004).

Variations of the torsion strain and elastic energy within the optimal regime of processive capping that was determined by our analysis (see supplemental material) are presented in Fig. 2. We found that immediately after the pointed end is immobilized, torsion stresses start to develop within the system, and the first steps of polymerization proceed in the stair-stepping mode, resulting in accumulation of a positive torsion strain. When the strain reaches the level of $\sim 83^\circ$, which corresponds to six stair-stepping steps, the screw mode becomes energetically more favorable. The following step is performed in the screw mode, which relaxes the previously accumulated strain and induces a negative strain of approximately -83° . Further processive capping consists of repeating sequences of ~ 12 stair-stepping steps followed by one screw step. Within each sequence, the torsion strain varies between approximately -83° and $\sim 83^\circ$ (Fig. 2 a). Periodic relaxation of the deformation, which does not allow the absolute value of the torsion strain to exceed $\sim 83^\circ$, prevents supercoiling. Indeed, the torsion strain that must be reached in order to generate supercoiling can be determined through the torsion (C) and bending (K) moduli of the actin filament, according to the relationship $\tau^* = \alpha \times \frac{K}{C}$, where $\alpha = 8.98$ is a numeric constant resulting from elastic analysis (Landau and Lifshitz, 1959). By using the aforementioned values of elastic moduli, we obtain that $\tau^* \approx 230^\circ$. Therefore, within the suggested regime of processive capping, the torsion strain always remains smaller than the critical value τ^* , and supercoiling is not expected, which is in agreement with the experimental observation.

The torsion elastic energy that accumulates within the system changes periodically, and the amplitude of this variation decreases slowly (Fig. 2 b). For the parameters that we have used, the maximum value of elastic energy is $\sim 20 k_B T$ (where $k_B T \approx 0.6$ kcal/M is the product of the Boltzmann constant and absolute temperature). Such a torsion energy is close to the energy of protein–protein interaction in the actin–formin system (Kozlov and Bershadsky, 2004) and, hence, appears to be feasible. At the same time, this torsion energy is high enough that it could significantly influence the effective actin–FH2-binding energy underlying the kinetics of stair-stepping and screw modes of processive capping.

The two proposed modes of processive capping differ substantially in terms of the intermediate conformations that the system has to pass on its way from the closed to the open state. These largely unknown factors may determine the relative kinetics of the two modes, whose detailed analysis will be performed elsewhere. In this study, we briefly discuss the most important related issues.

The screw step can be divided into substages. At the first substage, the torsion strain that accumulated during the sequence of stair-stepping steps relaxes to zero, and the system reaches an unstressed state of vanishing elastic energy. In this intermediate state, the filament barbed end is turned with respect to formin in such a way that terminal actin subunits can-

not bind to the formin dimer. To reassociate with the FH2 dimer and absorb a new actin monomer, the barbed end has to rotate with respect to formin by another $\sim 83^\circ$, which constitutes the next substage of the screw step. This rotation, which is followed by binding, results in accumulation of the torsion elastic energy but allows for release of the actin–formin- and actin–actin-binding energies. The resulting overall energy balance of the screw step is negative, meaning that it is favorable energetically. At the same time, before binding energy is released, the system has to overcome an energy barrier that is produced by the accumulating torsion strain. Note that the energy barrier of the same origin also exists at a stair-stepping step of processive capping. Indeed, every stair-stepping step is accompanied by an $\sim 14^\circ$ torsion strain and accumulation of the corresponding elastic energy. These elastic energy barriers may contribute to or even determine the kinetics of screw and stair-stepping modes. For the stair-stepping mode, the torsion strain and, hence, the elastic energy barrier are smaller; thus, the related rate limitation should be less significant.

Another origin of kinetic limitations of the two modes of processive capping is determined by the energy barriers that are related to transient detachment of formin bridge elements from the filament barbed end. The stair-stepping mode assumes that in the course of transition from the closed to the open state, only one FH2 bridge detaches from the barbed end, whereas the second bridge remains bound and does not move (Fig. 1 b). Thus, the stair step requires the dissociation of two actin–FH2 interaction sites (Otomo et al., 2005). The screw mode most probably requires simultaneous detachment of the two bridges from the four actin-binding sites in the closed state, because the tethers connecting the two bridges within an FH2 dimer appear to be not long enough to allow for sequential detachment of the bridges. This is followed by slipping of the two bridges around the filament toward the new position in the open state, where they are again trapped in the corresponding binding sites. The decision of the system to adopt one mode over the other must be strongly influenced by strain. In the absence of strain, the release of only a single bridge element should be significantly more probable than the simultaneous release of both bridge elements. Thus, under stress-free conditions (e.g., when the system is free in solution), stair stepping should be the more probable mode of procession. However, in the presence of torsion strain, binding interactions will be effectively weakened, which enhances the simultaneous dissociation of both bridges and facilitates the screw mode. Under positive torsion strain, the screw mode relieves strain and, therefore, is favored. Under negative torsion strain, stair stepping is favored for the same reason.

The existence of energy barriers, which can limit kinetics of the two modes of processive capping, results from consideration of the minimal system that consists of the barbed end and FH2 dimer. It is possible that overcoming these energy barriers and the acceleration of processive capping are caused by the active participation of FH1 and profilin (Romero et al., 2004). Indeed, the FH1–FH2 complex in the presence of profilin has been suggested to act as a processive motor, as it increases the barbed end polymerization rate (Romero et al., 2004).

Detachment in all four binding sites means that within the screw mode, the formin dimer transiently loses its specific interactions with the barbed end until it reaches three actin-binding sites in the open state. It is important to emphasize that this detachment does not necessarily mean complete separation of formin from the actin filament. Indeed, according to the structure of the formin–actin complex (Otomo et al., 2005) and the present model, the terminal subunits of the barbed end penetrate the formin ring in both the open and closed state of the system (Fig. 1). Thus, complete separation of formin from the barbed end requires, in addition to the bond detachment, axial translation of the formin ring off barbed end terminal subunits. In addition, if rotation occurs rapidly, the formin could remain associated with the filament through essentially nonspecific contacts during the screw transition. In experimentally relevant situations, the complete formin–actin separation was not expected, which is in agreement with observations of a low formin–barbed end dissociation rate (Zigmond et al., 2003; Kovar and Pollard, 2004). Indeed, as previously mentioned in this section, in situations in which formin is not immobilized on any substrate or membrane (Zigmond et al., 2003), the screw mode and, hence, the related simultaneous unbinding of two formin bridges from the barbed end are not expected. In the case of formin that is fixed on the substrate (Kovar and Pollard, 2004), actin polymerization produces a force pushing the barbed end against the formin cap. This force keeps barbed end terminal subunits within the formin ring, and separation between the latter is sterically prevented. In addition, electrostatic interaction between the highly positively charged post site and linker of FH2 and highly negatively charged actin groups should provide long-range interprotein attraction. This could help retain formin on the barbed end during rotational movement in the screw mode and lead it into the correct position that corresponds to the new bound state in the open configuration.

Another possibility could be that, as a result of the release of four bonds, the FH2 ring slides along the filament toward the filament bulk and binds behind the barbed end. This would expose an uncapped barbed end to actin monomer solution and allow for unconstrained polymerization. Such a scenario is prevented by distortion of the filament structure by the FH2 ring (Otomo et al., 2005), which is minimized when the ring is located at the very end of the filament. An effective force that is generated by elastic stresses retains the FH2 ring at the barbed end and prevents a free filament growth.

To summarize, we suggest that torsion elastic stresses determine the optimal regime of actin polymerization upon processive capping by a fastened FH2 dimer. This regime consists, on average, of repeating sequences of 12 stair-stepping steps followed by one screw step. Our model explains the unique properties of formin that enable it to generate arrays of actin filaments whose ends are firmly attached to other cellular structures.

Materials and methods

Online supplemental material

Online supplemental material describes a torsion elastic energy analysis of the process of actin filament polymerization upon processive capping by a

formin dimer. We consider a general case of torsion stress distribution between the actin filament and a link between formin cap and substrate. Based on this analysis, we determine an optimal regime of processive capping and find the corresponding variations of the torsion strain and elastic energy. We also show that within the optimal regime, supercoiling of the filament is not expected. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.200504156/DC1>.

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