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Active compaction of crosslinked driven filament networks*

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Abstract

The contractile ability of active materials relies on the interplay of force-exerting and force-bearing structures. However, the complexity of interactions and limited parameter control of many model systems are major obstacles in advancing our understanding of the underlying fundamental principles. To shed light on these principles we introduce and analyse a minimal reconstituted system, consisting of highly concentrated actin filaments that are crosslinked by α -actinin and actively transported in the two-dimensional geometry of a motility assay. This minimal system actively compacts and evolves into highly compact fibres that exceed the length of the individual filaments by two orders of magnitude. We identify the interplay between active transport and crosslinking to be responsible for the observed active compaction. This enables us to control the structure and the length scale of active compaction.

1 Introduction

The functionality of most biological systems relies on the self-organization of their constituents into higher-order structures. By the cost of constant dissipation of energy complex structures on all different length scales self-organize, ranging from the appearance of flocks down to the molecular scale in intracellular structures. One such self-organized structure is the contractile machinery of eukaryotic cells that is essential for numerous cellular processes ranging from the formation of stress fibers [1] and contractile rings during cytokinesis [2,3], to cell migration [4,5] and intracellular transport processes [6]. Conceptually, active materials like these owe their unique dynamic properties to the competition between force-exerting components like molecular motors and force-dissipating structures in the viscoelastic environment [7–11]. Microscopically, the contractile machinery is made up of two key components: actin and myosin II [12]. The contractile ability of the actin-myosin complex arises from crosslinked actin networks that canalize and transmit motor-mediated forces and translate them into contractile activity [13,14].

To identify the role of myosin in the self-organization processes, various studies have been devoted to simplified systems of purified cytoskeletal components [15–21]. In most of these reconstituted systems it was possible to relate the macro- or mesoscopic structure formation processes to the simultaneous action of myosin and crosslinking molecules and the competition between active driving and passive crosslinking. Recently, the reconstitution of contractile bundles in the presence of only myosin II filaments was achieved using a pre-patterning approach with functionalized colloidal beads [20].

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However, the direct reorganization processes of the individual components were rarely accessible and the self-organization principles and the mechanisms setting the size in these fascinating structures remain unresolved. This can certainly be attributed to the inherent complexity of such model systems, but also to the commonly used approach of the simultaneous action of molecular motors and crosslinking proteins: in a 3D system, the myosin II filaments do not only provide the active driving, but they themselves are simultaneously reorganized in the course of the structure formation process [16,17].

Only by a complete decoupling of the active driving from the actual structure formation process, it is possible to elucidate the underlying microscopic mechanisms and to assess the behaviour of crosslinkers in an active environment specifically.

To this end, we introduce high-density motility assay experiments, where highly concentrated actin filaments in the presence of crosslinking proteins are propelled by motor proteins that are immobilized on a surface. With this setup we show that a homogeneous α -actinin/actin network that is set under stress by molecular motors is intrinsically unstable. It disintegrates and forms actively compacting patches that finally evolve into highly compact α -actinin/actin fibres that exceed the length of individual filaments by two orders of magnitude.

The structure formation directly depends on the initial phase, where the reorganization of individual filaments leads to a phase separation between dense and dilute regions. The efficiency of this reorganization process and hence the length scale of the phase separation directly depend on the motor activity. This allows us to control the size and morphology of the α -actinin/actin fibres: high motor activities lead to fibres of up to 100 μm in length whereas low motor activities result in only 5 μm long fibres.

2 Materials and methods

2.1 Protein purification

G-actin was obtained from rabbit skeletal muscle (obtained from Hasenhof Weh, Albertshofen, Germany) following a standardized protocol [22,23]. Actin polymerization was initiated by adding one-tenth of the sample volume of a tenfold concentrated F-buffer (20 mM Tris, 20 mM MgCl_2 , 2 mM CaCl_2 , 2 mM DTT and 1 M KCl). To observe the dynamic pattern formation in the system, we added fluorescently labelled reporter filaments at a ratio of labelled-to-unlabelled filaments of 1:45 (if not stated otherwise). Reporter filaments were stabilized with Alexa-Fluor-488-phalloidin (Invitrogen) at a ratio of phalloidin to actin monomer of 1:2. Unlabelled filaments are stabilized with phalloidin (Sigma) likewise at a ratio of 1:2. Once polymerized, actin was used within 2 days. HMM is prepared from myosin II obtained from rabbit skeletal muscle following a standardized protocol [24]. The Michaelis-Menten constant for the used HMM preparation was determined to be $k_M = (98.9 \pm 12.3) \mu\text{M}$. α -actinin is isolated from turkey gizzard smooth muscle following ref. [25], dialyzed against G-buffer and stored at 4 °C for several weeks.

2.2 Sample preparation

Flow chambers were prepared with pre-cleaned microscope slides (Carl Roth, Germany) and coverslips (Carl Roth, Germany, 24 × 24 mm, No. 1). Coverslips were coated with a 0.1% nitrocellulose solution diluted from a 2% solution (Electron Microscopy Sciences, Hatfield, PA) in iso-amyl alcohol (Carl Roth, Germany). The coverslips were fixed to the microscope slides using parafilm, yielding an overall chamber volume of $\sim 30 \mu\text{L}$. Prior to each experiment, a 30 μL actin dilution with 7.6 μM was prepared by gently mixing labelled and unlabelled actin filaments with Assay Buffer (25 mM Imidazolhydrochlorid pH 7.4; 25 mM KCl; 4 mM MgCl_2 ; 1 mM EGTA; 1 mM DTT). The flow chamber was incubated with

HMM diluted in Assay Buffer. Prior to the insertion of the respective actin dilutions, surfaces are passified with BSA (1 mg/ml BSA (Sigma) dissolved in Assay Buffer). To start the experiment, ATP (Sigma) together with the respective crosslinker concentration dissolved in Assay Buffer (50 μM) is flushed into the flow chamber. The experiments were performed under the addition of an ATP regeneration system using creatine phosphate and creatine phosphate kinase to keep the ATP concentration constant. Oxidation of the fluorophore was prevented by adding a standard antioxidant buffer supplement GOC (2 mg Glucose-Oxidase, Sigma; 0.5 mg Catalase, Fluka). To verify that the observed patterns are not affected by the use of GOC, control assays without the antioxidant supplement were performed. After the addition of the Motility Buffer, the flow chambers were sealed with vacuum grease (Bayer Silicones).

2.3 Image acquisition, preprocessing and evaluation of the meshsize

All data are acquired on a Leica DMI 6000 inverted microscope with a $\times 63$ oil objective (numerical aperture: 1.4) or a $\times 40$ oil objective (numerical aperture: 1.35). Images (resolution: 1344×1024 pixels) were captured with a charge-coupled device camera (C4880-80, Hamamatsu) attached via a 0.35 or 1.0 camera mount. Image acquisition and storage are carried out with the image processing software “OpenBox” [26]. Prior to the mesh size analysis, a background subtraction is performed using ImageJ (rolling ball radius of 250 pixels). For the Kymographs the ImageJ-Plugin of J. Rietdorf and A. Seitz was used. For the calculation of the meshsize of the evolving structures, the image is converted into a binary image with the same threshold for all images. Subsequently, the discrete derivative in x - in y -direction is calculated. For each direction the distance between rising flanks is measured. A cumulative histogram over all distances is generated and fitted using a single exponential $e^{-x/\lambda}$. To avoid errors, only the histogram entries between 20% and 80% of the maximal value are considered. The mean over λ for the x - and y -direction is the average mesh size [27].

2.4 Actin, crosslinker and HMM concentrations

The numbers for the actin concentration c_a denote the monomeric actin concentration inserted into the flow chamber. This concentration is slightly lowered by up to 20% by the subsequent rinse with the ATP-crosslinker dilution (as can be inferred from the decline in the bulk fluorescence intensity measured by a $\times 20$ objective). The crosslinker concentration denotes the concentration in the bulk fluid. For all experiments the flow chamber was incubated with 1.8 μM of HMM dissolved in Assay Buffer.

2.5 Particle Image Velocimetry (PIV)

The velocity profiles were calculated with a customized Particle Image Velocity algorithm written in MATLAB. The PIV code estimates the most probable displacement of small quadratic blocks by evaluating the cross-correlation function of two consecutive images [28]. For all images a blocksize of 32 pixels was used.

2.6 Measurement of the filament length

Following the approach of Schmoller *et al.* [29], we used the MATLAB function “regionprops” to identify the interconnected objects with an area of > 10 pixels. Subsequently, the objects are fitted with an ellipse that has the same normalized second central moments as the region, and the major axis length is used as a measure for filament length. The usage of the “regionprops” library requires the conversion of the microscopy images into binary images. This was carried out in ImageJ with a threshold adjusted globally for all images. Distributions are obtained from a minimum of 10 images.

3 Phase behaviour and active compaction

To shed light on the principles that underlie the self-organization of systems that are actively driven and at the same time are subjected to passive crosslinking, we use a high-density motility assay [30,31]. The system consists of actin filaments that are propelled by non-processive motor proteins (HMM) in the planar geometry of a standard motility assay. In the regime of high actin densities and without crosslinking proteins, the filaments self-organize to form coherently moving structures, clusters and density waves [31]. This drastically changes if the crosslinking protein γ -actinin is added to the motility assay.

γ -actinin is highly abundant in contractile cytoskeletal assemblies like myofibrils, stress fibres and contractile rings [2,32–34]. It is a promiscuous crosslinker that connects neighbouring filaments regardless of their orientation, thus leading to the formation of predominately apolar structures [35]. By that, the crosslinker directly opposes the active transport in the motility assay that relies on the structural polarity of the filaments. Initially, this leads to a homogeneous distribution of actin filaments that are firmly incorporated in a two dimensional γ -actinin/actin network (fig. 1(c)). This elastic network is actively set under stress by the underlying motor proteins.

The interplay of γ -actinin crosslinkers and the motor activity determines the further dynamic development of this initial basic state. Consequently, the phase behaviour of the system depends on the γ -actinin concentration c_{aa} (fig. 1). For a given actin concentration of $c_a = 7.6 \mu\text{M}$, high γ -actinin concentrations above $c_{aa} = 0.23 \mu\text{M}$ effectively prevent any further reorganization and the homogeneous basic state is frozen in (fig. 1(c)). In this regime of high crosslinker concentrations, the motor proteins are not able to induce large-scale rearrangements in the network. On the other extreme, in the regime of low γ -actinin concentrations below $c_{aa} = 0.05 \mu\text{M}$, the amount of crosslinking proteins in the network is too small to sustain the integrity of the network and the motor proteins drive the active fluidization and the initial basic state disintegrates (fig. 1(a)). Subsequently, the system self-organizes into coherently moving structures reminiscent to the collective motion observed in the motility assay without crosslinks (supplementary movie M1). Yet, even in this concentration regime, the influence of crosslinkers is still visible as the competition between motor-induced active transport and passive crosslinking leads to a shortening of the filaments. Moreover, the density inhomogeneities that characterize the high-density motility assay without crosslinkers are less pronounced. Only for trace amounts of γ -actinin below $c_{aa} = 0.005 \mu\text{M}$, the phase behaviour of the high-density motility assay without crosslinkers can be retrieved —exemplifying the high sensibility of the setup towards the addition of crosslinking proteins.

For any given actin concentration there exists an intermediate γ -actinin concentration, where the motor activity and the influence of the crosslinkers are relatively balanced. This allows the active reorganization of the initial basic state and the same time ensures the integrity of the network. This is mandatory for the subsequent dynamic structure formation that leads to the self-organized formation of highly compact γ -actinin/actin fibres (fig. 1(b)).

The ensuing self-organization process thereby can be conceived as a phase separation between patches that are densely packed with material and depleted regions that appear as holes in the γ -actinin/actin microstructure (fig. 2(a)-(f) and supplementary movie M2). While filaments in the dense regions are firmly crosslinked and barely move, individual filaments in the depleted regions can move freely and perform persistent random walks before they get crosslinked again. Successively, the dense patches contract and eventually evolve into the compact actin/ γ -actinin fibres observed in the steady state, reflecting the tendency of γ -actinin to form bundles in passive *in vitro* networks [36].

The self-organization takes place on the timescale of several tens of minutes in a multistage coarsening process (fig. 2(b)-(e) and supplementary movie M2). As can be inferred from the area fraction covered by the dense patches (fig. 3), the process of active compaction is continuous and lasts for 30 min, until a steady state of maximal compaction is reached.

In contrast to other reconstituted systems, the observed active compaction neither relies on the formation of contractile foci of aggregating motor proteins nor on the pre-assembly of F-actin bundles. The isotropic active driving of immobilized motor proteins is sufficient to destabilize the crosslinked F-actin network and to trigger the self-organized formation of compact fibres.

4 The multiple stages of active compaction

The active compaction in the system can be further quantified by introducing the average mesh size of the densely packed regions as order parameter (for details see the section “Materials and methods”). Starting from the initial, homogeneously distributed state with a mesh size of $\approx 8 \mu\text{m}$, increases monotonically with time until the steady state is reached that averages a meshsize of $\sim 50 \mu\text{m}$ (fig. 3(a)). The overall temporal evolution of the order parameter is characterized by subtle variations in the rate of change of $\langle \ell \rangle$: In the early stage of the pattern formation, a linear rise in the mesh size occurs. A plateau in which the mesh size stays almost constant follows it. In the late stages of the pattern formation the coarsening process gets increasingly slower, before it finally levels off again in the steady state. These variations in the rate of change correspond to the distinct stages of the observed multistage coarsening process. The different stages are: A) an active shortening of the filaments and the dynamic reorganization of the fragments that triggers the formation of densely packed patches; B) the active transport of entire patches; C) the disintegration of the dense patches and D) their internal compaction.

4.1 Active shortening of the filaments and reorganization of the fragments

In the initial stage of the multistage coarsening process, the initially $10 \mu\text{m}$ long isotropically distributed filaments are ruptured into $1 \mu\text{m}$ fragments (fig. 4 (a) and (b) and supplementary movie M3). This results from the competitive binding of molecular motors and passive crosslinkers to the actin filaments. The active fragmentation is the precondition for the subsequent reorganization of the filaments: only filaments that are short enough can readily detach from the network and can be actively transported, while longer filaments stay firmly crosslinked to the network (fig. 4(c)). This is reminiscent to observations in cellular systems where the structure formation also requires predominantly short actin filaments with a highly conserved filament length below $1 \mu\text{m}$ as found for example in sarcomeres.

The dynamic reorganization of the filament fragments is the microscopic cause for the observed phase separation of the network into dense and dilute regions (supplementary movie M2). The fragments detach more easily from regions where the material density and thus the number of possible binding partners is low. On the other hand, they are prone to get bound again at regions where the material density is high. This is sufficient to destabilize the α -actinin/actin network as local density inhomogeneities are amplified. Successively, this leads to the observed phase separation into dense patches and dilute zones.

4.2 Active transport of entire patches

In the next stage, these patches are transported as a whole by the underlying motor proteins (fig. 3 and supplementary movie M2). The patches have a size of the order of 10 to $100 \mu\text{m}^2$. Once formed, they are stable and move on straight and persistent trajectories with an average velocity of up to $0.1 \mu\text{m/s}$, one order of magnitude slower than the gliding speed of individual filaments (fig. 5). The movement of the different patches is uncorrelated in

direction and velocity. In the quasi-two-dimensional geometry of the motility assay, this leads to a further condensation, as the uncorrelated movements of the patches results in the formation of spots where different patches converge and material gets accumulated (fig. 5(b)). Despite the slow velocity of the moving area fractions, this process results in a comparably rapid compaction that lasts for around 15 min (fig. 3).

4.3 Disintegration of the dense patches

That followed, in the disintegration phase, the condensation speed starts to decrease because the phase separation into densely packed patches and dilute zones is almost completed. Any further condensation is only possible if the network disintegrates by a rupturing of the dense patches (fig. 6 and supplementary movie M4). This leads to the formation of isolated patches. Once a rupturing event occurs, patches move freely on straight paths, until they collide and merge with other patches. The rupturing results in the stagnation of the average mesh size since the compaction is compensated by the formation of new holes (fig. 3 and fig. 6 and supplementary movie M4).

This is accompanied by a change in the morphology of the structures during the disintegration phase: Because patches are not firmly interconnected with other patches, they are able to flow past each other, leading to the emergence of zigzag-like patterns (fig. 2(c) and (d) and supplementary movie M2). Crosslinking of converging patches leads to larger structures. Successively, highly condensed structures form. These are not transported any more and the active transport now fully contributes to their compaction. This comparatively rapid process is recovered in the steep increase in the mesh size at the end of the disintegration phase.

4.4 Internal compaction

After a time period of 30 min, the α -actinin/actin network has entirely disintegrated into isolated and non-moving structures. This marks the beginning of the phase of internal compaction, where any further decrease in meshsize is mainly based on reorganization processes within the structures, resulting in highly compact, yet immobile fibres (fig. 7). This is recovered in a slow increase in the average mesh size.

5 The role of the polarity for the structure formation

While the dynamics in the initial phase relies on the reorganization of individual filaments, the rapid compaction in the subsequent stages of the coarsening process requires the active transport of entire patches (fig. 3(b)). Remarkably, the active transport of the patches is highly persistent both in direction and velocity and as long as the patches do not interfere with neighbouring structures, they move on straight trajectories (fig. 5 and supplemental movie M2).

The active transport of entire patches is likely to rely on a non-vanishing net polarity of the patches, providing the small but stable directional bias necessary for the observed dynamics. If the patches had a vanishing net polarity with filaments that are oriented randomly, any motor-induced active transport would result in a displacement in a random direction. Most likely these displacements would cancel out and the patch either would not move at all, or would show a highly non-persistent motion with frequent directional changes and a varying velocity.

To further explore this aspect, we performed a set of experiments where we let the system evolve starting from a basic state that is polar on large length scales. In the motility assay such polar basic states can readily be prepared by allowing the system to self-organize into coherently moving structures before crosslinking proteins are added. The polarity of the

basic state is then determined by the F-actin concentration: high actin concentrations yield coherently moving structures with a high polarity, while lower actin concentrations result in smaller polar domains (fig. 8 (a) and (d)).

If the dynamics of the patches indeed depended on the net polarity of the patches, a higher initial polarity should have two main consequences: an increase of the patch size and an increase of the overall structure size in the steady state. As can be seen from the patch dynamics during the pattern formation (fig. 8 (b) and (e)) and the steady-state images (fig. 8 (c) and (f)), this is exactly the case: the higher the polarity of the initial basic state, the higher is the patch size and the bigger is the size of the α -actinin/actin fibres in the steady state (also see supplemental movie M5). The higher polarity of the patches also becomes manifest in a higher gliding velocity: patches that emerge from a polar basic state move with velocities of up to $0.25 \mu\text{m/s}$, significantly higher than observed for apolar starting conditions.

6 Controlling the length scale of active compaction

The complex dynamics in the present system include the active transport of entire patches, rupturing events and the internal compaction of the emergent structures (fig. 3). The precondition for all this is reorganization and relocation of individual filaments that trigger the formation of the dense patches. Microscopically, this reorganization is comparatively simple: it is based on the detachment of single filament fragments that then are actively transported by the motor proteins before they get bound again. This competition between active transport and passive binding is governed by three microscopic parameters: the effective filament (un-)binding rates k_{off} and k_{on} and the motor-induced sliding velocity of individual filaments v . Together, these parameters set the length scale of the observed phase separation during the initial stages of the pattern forming instability: The length scale is limited by the average distance $\langle x \rangle$ individual filaments can cover after having detached from the network. This average distance is determined by the average detachment time of an individual filament $\sim k_{\text{off}}/(k_{\text{off}} + k_{\text{on}})$ and the gliding speed v according to $\langle x \rangle \sim vk_{\text{off}}/(k_{\text{off}} + k_{\text{on}})$. The rates k_{off} and k_{on} denote the effective on- and off-rates of an entire filament. They depend on the local filament and crosslinker densities and the crosslinker on- and off-rates under load, when the system is actively set under stress by motor proteins.

Providing an estimate for the length scale of phase separation during the initial stages of the pattern formation, the above expression for the average gliding distance $\langle x \rangle$ bears two important implications. First, since it directly depends on the filament velocity v , any finite filament velocity should result in a phase separation. And, second, the structure size in general should be controllable by the filament gliding velocity. In the experiment this can readily be addressed by varying the ATP concentration (c_{ATP}) as it sets the filament speed according to the Michaelis-Menten kinetics $v \sim c_{\text{ATP}}/(k_{\text{M}} + c_{\text{ATP}})$ with k_{M} being the enzyme-specific Michaelis-Menten constant. For small ATP concentrations v rises linearly with c_{ATP} and approaches a maximal saturation velocity v_{max} for the freely moving filaments. Moreover, the ATP concentration affects the crossbridge strength of the acto-myosin complex. Lowering the ATP concentration leads to higher binding affinity of the myosin heads to the actin filaments. This effect decreases the effective off rate k_{off} and thereby can be expected to further decrease the length scale $\langle x \rangle$ of the phase separation.

As can be seen in the steady-state images (fig. 9(a)-(c)), the structure size is indeed tightly regulated by the ATP concentration. Small ATP concentrations ($1 \mu\text{M}$), corresponding to small gliding velocities, yield small structures with a length of up to $10 \mu\text{m}$ and a thickness of up to $5 \mu\text{m}$. For high ATP concentrations, corresponding to the maximal gliding velocity, considerably bigger structures emerge: here, the fibres have a length of up to several

hundreds of micrometers and a thickness of up to $10\ \mu\text{m}$. This ATP dependence of the structure size is also reflected in the cumulative length and thickness distributions (fig. 9(d)). The length distributions (fig. 9(d)) are of exponential shape with decreasing decay length for decreasing ATP concentrations. Likewise, a decrease of the ATP concentration leads to a narrower thickness distribution. Altogether, the elongated fibres that evolve at high ATP concentrations incorporate up to 100 times more filaments than the ones that form in the presence of low ATP concentrations. This exemplifies the much higher level of compaction for high ATP concentrations.

The morphology of the fibres is intimately related to the mesh size $|\lambda|$ observed in the steady state. The higher the level of compaction, the more material is concentrated on fewer but larger fibres. By consequence, the average spacing between the fibres and thus the mesh size $|\lambda|$ increase with increasing ATP concentration. Starting from a minimal mesh size of $|\lambda| = 10\ \mu\text{m}$ at low ATP, $|\lambda|$ increases with increasing ATP concentration until it saturates at an average mesh size of $|\lambda| = 60\ \mu\text{m}$ (fig. 9(e)). The saturation correlates to the saturation of the filament gliding velocity at v_{max} for high ATP concentrations (fig. 9(e)) —further confirming the microscopic picture of the balance between active transport and passive binding that sets the length scale of contraction in the initial stage of the pattern formation.

Remarkably, the structure formation in the system does not require a critical ATP concentration. For any finite ATP concentration or filament gliding velocity, the homogeneous α -actinin/actin network is unstable and disintegrates into dense patches—in full agreement with the microscopic picture. By contrast, the initial network is stable when no ATP is supplied.

Accordingly, the motor activity constitutes an effective switch which allows the system to adjust the length scale of the structure formation: A high motor activity yields a pattern formation that is coordinated over a distance of more than $50\ \mu\text{m}$, exceeding the size of the individual filament by two orders of magnitude. By contrast, low ATP concentrations result in small patterns with a diameter in the order of the filament length. The ATP concentration also controls the aspect ratio of the structures: while high ATP concentrations lead to highly elongated fibres, low ATP concentrations lead to more rounded structures (fig. 9(a)-(c)). This motor-activity-dependent formation of condensed structures is reminiscent to the force-dependent aggregation of α -actinin/actin networks in rheological experiments [37].

7 Discussion

The active compaction of the two-dimensional α -actinin/actin network results from the interplay between active transport and the ability of α -actinin to crosslink filaments regardless of their orientation: This interplay can be controlled by varying the relative crosslinker concentration and the motor activity. Especially the motor activity constitutes an effective switch that allows active systems to tailor the size of the emerging structures: while a low activity yields small structures, a high activity results in larger structures. This makes the motor activity to an effective and powerful tool to steer the self-organized assembly and the length scale of the contractile machinery in active matter.

The comparison between α -actinin and other crosslinking proteins could provide another important insight in the self-organization of active systems: even if crosslinking proteins behave similar in passive systems, they may display a surprisingly different behaviour once they are set under stress in active systems. As the properties of active materials crucially depend on the behaviour of the crosslinking proteins, it is thus indispensable to explicitly address their behaviour in well-defined out-of-equilibrium systems either on the single-molecule level or in minimal systems like the one presented here. The high variability in the

behaviour of different crosslinking proteins in active systems adds a new degree of freedom to the physics of active gels. By varying the specific abundances of crosslinking proteins, active networks could not only tune their mechanic properties, but could also shift gears between different mechanisms and regimes of self-organization —and by that effectively react to changing needs.

The conditions for the acto-myosin interaction in motility assay experiments are similar to the conditions found in the lamellipodium of migrating cells [30]; for instance the average motor density applied here resembles the myosin concentration in platelets [38] and like the active networks investigated in this study, the lamellipodium is densely packed with actin filaments [39]. In the lamellipodium, however, actin filaments themselves are subjected to dynamic assembly and disassembly [40]. The extension of the model system to include F-actin (de-)polymerisation dynamics together with various crosslinking proteins offers a promising route for *in vitro* studies of the active turnover of contractile assemblies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Phase behavior as a function of the α -actinin concentration. For low crosslinker concentrations below $c_1 = 0.05 \mu\text{M}$, the steady state of the system is characterized by the collective movement of driven filaments similar to the high-density motility assay without crosslinking proteins. Compared to the case without crosslinkers, the structures are less pronounced as the crosslinking proteins dampen the formation of density inhomogeneities. Moreover, due to the competition between active driving and crosslinking, the filaments get actively ruptured. Increasing the crosslinker concentration above a threshold concentration of $c_1 = 0.05 \mu\text{M}$ leads to a fundamentally different behavior: instead of collectively moving structures, the system actively compacts and forms highly condensed actin/ α -actinin fibres (b). These fibres consist of millions of individual filaments and exceed the size of the individual filament by three orders of magnitude. The active compaction is only possible in an intermediate concentration regime: at high crosslinker concentrations above $c_2 = 0.23 \mu\text{M}$, the motor activity is not sufficient to disintegrate the network and the isotropic initial state is preserved (c). For (a) the α -actinin concentration was adjusted to $c_{aa} = 0.005 \mu\text{M}$, for (b) to $c_{aa} = 0.17 \mu\text{M}$ and for (c) to $c_{aa} = 0.46 \mu\text{M}$. The actin concentration was set to $7.6 \mu\text{M}$. All scalebars are $25 \mu\text{m}$.

Fig. 2.

An α -actinin/actin network that is set under stress by underlying molecular motors is unstable. Within several minutes it is disintegrated and driven into a phase separation between regions densely packed with material and dilute regions, where only a limited number of filaments can be found. These dilute regions appear as holes in the network microstructure (a) and (b). With time, the holes coarsen as the densely packed patches further compact (a)-(c). Yet, the dense patches are still interconnected. When the diameter of the holes approximately equals the diameter of the dense patches (c), any further compaction requires a rupturing of the dense patches (d). This leads to the successive formation of isolated patches (e). Any further compaction is then based on internal reorganizations of these isolated patches (e) and (f). In the steady state of the pattern forming instability, the system consists of isolated tightly bundled patches (f). The actin concentration is $7.6 \mu\text{M}$ and the α -actinin concentration $0.17 \mu\text{M}$. All scalebars are $25 \mu\text{m}$.

Fig. 3.

The active compaction of the γ -actinin/actin network undergoes distinct stages in a multistage coarsening process. This is visible in the continuous decline of the occupied area fraction and the synchronous increase in the mesh size of the network (a). Each phase is characterized by distinct microscopic processes that are schematically depicted in (b). In the initial phase, the actin filaments are ruptured into approximately $1\ \mu\text{m}$ long fragments. The fragmentation is the precondition for the subsequent reorganization of the system that is based on the competition between passive binding and active transport of individual filaments. The relocation of individual filaments leads to the phase separation of the network into dilute and densely packed patches. The active transport of entire patches marks the beginning of a phase of rapid compaction that drives the disintegration of the network, visible in the linear decline in the occupied area fraction and the concomitant decrease in mesh size. This compaction continues until the size of the dilute regions and the dense patches are approximately equal. Now, any further compaction requires a rupturing of the patches, further disintegrating the network. On the other hand, freely moving patches collide and coalesce leading to tightly interconnected non-moving structures. This is reflected in a decline in the slope of the occupied area fraction and a plateau in the mesh size. This stage that is governed by the reorganization of entire patches continues until the entire network has disintegrated into isolated non-moving structures. Now, any further compaction is mainly based on the internal compaction of these structures, leading to the compact γ -actinin/actin bundles observed in the steady state. The actin concentration is $7.6\ \mu\text{M}$ and the γ -actinin concentration $0.17\ \mu\text{M}$.

Fig. 4.

Filament rupturing and filament length distributions. The competition between active transport and crosslinking leads to a rapid fragmentation of individual filaments. The image series in (a) shows the motor-induced fragmentation of a filament that gets bound to the α -actinin/actin network at $t = 0$. Within several seconds, the initially 20 μm long filament gets ruptured into subfragments not larger than 5 μm in length. The motor-induced rupturing also becomes manifest in the overall cumulative filament length distributions $P(l)$ shown in (b). Before α -actinin is added, the filament lengths are broadly distributed (black curve). After the addition of α -actinin the distribution considerably narrows as the filaments get ruptured (gray curve). The rupturing of individual filaments is the precondition for their subsequent dynamic relocation, as can be seen in the length distributions $p(l)$ shown in (c). While long filaments are prone to be firmly crosslinked, shorter filaments can readily detach from the network and are actively transported. The actin concentration is 7.6 μM and the α -actinin concentration 0.17 μM . The scale bars are 10 μm .

Fig. 5.

The dynamic compaction of dense patches relies on the convergence of individual patches. Image (a) shows the active compaction of a dense patch over a time period of 250 s (thin line: initial position; bold line: final position). The compaction of the patch relies on the active transport of subdomains that move independently of one another. This can be seen in the kymographs along two cross-sections of the patch shown in (b) and (c). While (b) shows the homogenous compaction around a zone where several small subdomains converge, (c) illustrates the displacement of an entire subdomain. The size of the polar subdomains that drive the compaction can be inferred from their velocities (d) and (e). In (b) the compaction is mediated by subdomains that have a diameter of approximately $10\ \mu\text{m}$, as exemplified by the regions marked with I and II in (b) and (d), respectively. In (c) the movement is mediated by a subdomain that is $100\ \mu\text{m}$ in diameter. This is reflected in a constant velocity in the entire field of view (e). All scale bars are $25\ \mu\text{m}$. The α -actinin concentration is $0.22\ \mu\text{M}$ and the actin concentration is $16\ \mu\text{M}$.

Fig. 6.

In the disintegration phase of the pattern forming instability, the further compaction of the systems requires the rupturing of dense patches. This is illustrated in the image series shown in (a)-(d) over a time period of 375 s and the associated kymograph (e). In image (a) the further compaction is hampered by the connectivity of the dense patches. The underlying motor proteins build up tension that finally leads to rupturing of the dense patch at its weakest point (b). This is followed by the free retraction of the patch (c) with an average retraction speed of $0.07 \mu\text{m/s}$ (e). The free retraction continues until the tip of the patch hits another patch to form a new zone of convergence (d). This is reflected in the subsequent decline in the retraction speed to $0.05 \mu\text{m/s}$. All scale bars are $10 \mu\text{m}$. The actin concentration is $7.6 \mu\text{M}$ and the α -actinin concentration $0.17 \mu\text{M}$.

Fig. 7.

Once the network has disintegrated into isolated patches, any further contraction relies on the relatively slow internal compaction within the isolated patches. The figure exemplarily shows the temporal evolution of the cross-section of such an isolated patch. Within 1500 s it compacts from a thickness of $6\ \mu\text{m}$ (FWHM) to a thickness of $2.5\ \mu\text{m}$ (FWHM). During the compaction, the total fluorescence intensity stays approximately constant, indicating that the observed compaction is not based on the loss of material. The actin concentration is $7.6\ \mu\text{M}$ and the α -actinin concentration $0.17\ \mu\text{M}$.

Fig. 8.

(Colour on-line) The addition of γ -actinin to a polar basic state yields larger and faster moving patches. Images (a) and (d) show microscopy images of collectively moving filaments at different filament densities in the absence of γ -actinin. High filament densities ($c_a = 10 \mu\text{M}$) lead to the formation of highly ordered collective motion patterns (a). The yellow arrows denote the velocity profile obtained by particle image velocimetry. The polar order of this basic state is conserved after γ -actinin is added (panel (b) and supplementary movie M5). Compared to the case with apolar starting conditions, the patches move with a fivefold faster velocity of $0.15\text{--}0.25 \mu\text{m/s}$ and the resulting patch size is drastically bigger. This can be seen in (b), where all velocity vectors belonging to coherently moving patches are grouped and coloured. Patches that move predominately to the right are coloured in red, patches that move to the left are marked in blue. The larger patch sizes for polar starting conditions also affect the properties of the steady state that shows considerably thicker and larger bundles (c). At lower F-actin concentrations ($5 \mu\text{M}$) the polarity of the initial basic state is considerably less pronounced (d). This leads to smaller patch sizes (e), slower moving patches ($0.10\text{--}0.15 \mu\text{m/s}$) and smaller structures in the steady state (f). The γ -actinin concentration was $0.17 \mu\text{M}$. For (a)–(c) a ratio of labelled to unlabelled filaments of 1:45 was used; for (d)–(f) the labelling ratio was adjusted to 1:20. In the absence of γ -actinin the average filament speed is $4.2 \mu\text{m/s}$.

Fig. 9.

The motor activity controls the morphology and the meshsize of the compact fibres. The images (a)-(c) show the steady state of the pattern formation for three different ATP concentrations. For low ATP concentrations, small fibres emerge; they have an average length l of up to 15 μm and exceed the size of the individual filament by one order of magnitude as can be seen from the cumulative distributions $P(l)$. The thickness d of these structures is limited to 5 μm (d). Higher ATP concentrations yield considerably bigger structures: with a length of up to several hundreds of micrometers, they exceed the length of the individual filament by two orders of magnitude. The growth in length is accompanied by a slight growth in thickness (d). For all ATP concentrations the fibres lengths are exponentially distributed. Altogether, fibres that were formed under high ATP concentrations incorporate considerably more material. The ATP concentration not only determines the morphology but also the intermediate spacing between individual fibres. This is retrieved in the average mesh size $|\lambda|$ that increases with increasing ATP concentration (e). Towards high ATP concentrations, $|\lambda|$ saturates at an average mesh size of 60 μm . For any finite ATP concentration, the homogeneous α -actinin-actin network is unstable and disintegrates into compact patches. By contrast, the initial network is stable when no ATP is supplied. All scale bars are 25 μm ; the actin concentration was set to 7.6 μM and the α -actinin concentration was set 0.17 μM .