Structure formation in active networks

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Supplemental Figures

**Figure S1:** Dynamic regime at high motor concentrations. (a) At of 1 μM actin, 1 μM fascin and 0.2 μM myosin 90 min after initiation of polymerization dynamic clusters form, as can be seen in the fluorescence micrograph. (b) The time overlay for 60–65 min after polymerization shows large rearrangements of these clusters. (c) Compared to lower myosin concentrations (0.1 μM, $\kappa = 0.1$), the cluster size decreases for higher myosin concentrations ($\kappa = 0.1$).
Figure S2: Time evolution of the cluster size distribution at different actin concentrations. (a) At low actin concentrations (0.5 μM), the cluster areas remain small and predominantly uniformly sized. (Inset) A significant amount of the cluster mass represented by their proportion to the total cluster area is retained in small (with area smaller than 15.8 μm²) and medium sized clusters (with area smaller than 31.6 μm²). (b) Numerous small clusters and only very few large clusters can be seen in the fluorescence micrograph in the steady state. (c) At higher actin concentrations (7.5 μM), the size of the large clusters becomes huge. This results in a single large cluster. (Inset) Accordingly, the mass is accumulated in this large cluster, while small (with area smaller than 100 μm²) and medium sized clusters (with area smaller than 794 μm²) contribute only very little in the total mass. (d) As can be seen in the fluorescence micrograph, only part of this huge, single cluster can be resolved in the microscope even at minimal magnification.
Figure S3: Mean square displacement (msd) for different actin concentrations. (a) The ensemble averaged mean square displacement in the transient state shows superdiffusion for all actin concentrations (0.5 µM blue circles, 1 µM cyan crosses, 2 µM green triangles, 7.5 µM red squares). (b) In the steady state, stalling events predominate at high actin concentrations (above 0.5 µM) resulting in a flatter msd. Colors are as in a. Solid black lines are power laws with indicated exponent to guide the eyes.
Figure S4: Temporal evolution of the cluster size distribution $\Pi$ obtained from the simulations. Like in the experiment, three different sizes can be defined. Initially the cluster size distribution decreases as a power law for small clusters and then exponentially for medium sized or larger clusters. With time, in the course of the clustering process, the probability of finding large clusters increases compared to the power law. The cluster size is measured in number of rods and the parameters are: $p_{off} = 0.02$, $p_{on} = 0.01$, $r_{off} = 0.01$, $r_{on} = 0.1$, $\rho = 1.4$. Single rods are not taken into account.
Movie captions

**Movie S1:** Structure formation in the quasi-static state. In the quasi-static state, actin/fascin/myosin-II networks show only local rearrangements. The overall structure is stable over time.

**Movie S2:** Structure formation in the dynamic state. In the dynamic state, active gels consisting of actin, the actin bundling protein fascin and myosin-II motor filaments exhibit drastic structural rearrangements. Over time, a coarsening process is observed due to cluster coalescence.

**Movie S3:** Dynamics of cluster formation. Initially in the transient state, small (blue) and medium sized (green) clusters predominate. These fuse to form an increasing number of large clusters (red). In the dynamic steady state, fusion and rupture events are balanced. Consequently, the mean cluster size and the relative number of small, medium or large clusters remain constant.

**Movie S4:** Time-lapse movie of simulation results. Starting from homogeneously distributed rods, distinct clusters are formed with time.
Simulation

To elucidate the microscopic processes that lead to the experimentally observed structure formation process we use a phenomenologic simulation that is based on probabilistic interaction rules. The microscopic interactions arise through the interplay between molecular motors and crosslinkers and include active transport, crosslinking or binding and forced unbinding that subsequently are described in detail.

**Passive and active binding processes.** If two rods overlap, passive binding events occur with a rate of $p_{on}$. Within each time step, after all overlaps have been rastered and checked for new passive binding sites, interconnected rods are pooled to clusters. If an overlap between two rods is not already occupied by a passive crosslinker, motor proteins can actively crosslink two intersecting rods with a rate $r_{on}$.

**Unbinding and forced unbinding.** All passive crosslinkers are subjected to unbinding events. Without the incorporation of forced unbinding, unbinding events occur at a rate $p_{off}$. For the phenomenological description of forced unbinding processes in the presence of motor proteins, the number $\epsilon$ of active crosslinks per cluster is determined. Since $\epsilon$ is proportional to the total stress exerted in the interconnected cluster, the averaged unbinding rate $p_{off,forced}$ should increase with $\epsilon$. Conceptually the stress increase leads to rupture events within the cluster whereby especially weakly crosslinked structures with a low actin and/or crosslinker density are prone to rupture.
In a simplified picture this can be modelled by increasing the off-rate with the number of motor proteins per individual cluster. With this approach an increased off-rate results in the predominant dissociation of weakly crosslinked structures within clustered structures – like it is observed experimentally.

In the simulations the increase in the off-rate as a function of the number of active crosslinks is described by the relation

\[
p_{\text{off, forced}} = p_{\text{off}} + \left( \frac{p_{\text{off, max}} - p_{\text{off}}}{\epsilon_{0.5} - \epsilon} \right) \cdot \epsilon,
\]

whereby \( p_{\text{off, max}} \) denotes the maximal off-rate due to forced unbinding and \( \epsilon_{0.5} \) is the number of crosslinks necessary to rise the original off-rate by \( 0.5 \cdot (p_{\text{off, max}} - p_{\text{off}}) \).

Noteworthy, an increased crosslinker off-rate does not necessarily compromise the overall stability of large clustered structures as the ultimate pullout of ruptured structures gets increasingly difficult the larger the clusters are; more likely, ruptured structures rebind in a different conformation. Consequently, an increased off-rate predominately leads to an increased degree of reorganization within large clusters.

**Active transport** If two actin fascin bundles are actively crosslinked, torques and forces are exerted by the bipolar motor filament walking towards the plus ends of the bundles. The velocities that arise from these active crosslinks can be calculated based on a microscopic force balance of two interacting rods. If \( \mathbf{r}_1(t) \) and \( \mathbf{r}_2(t) \) are the vectors to the center of mass of two intersecting rods (see Fig. S5), the position of the motor protein right
after the binding event at $t = 0$ is given by the Straley coordinates $^1$:

$$m_1(t = 0) = r_1 + \xi u_1, \quad m_2(t = 0) = r_2 + \eta u_2.$$  

If the motor moves with the velocity $v_m$, the motor position after a timestep $\Delta t$ is given by

$$m_1(t + \Delta t) = r_1(t) + (\xi + v_m \Delta t)u_1, \quad (2)$$
$$m_2(t + \Delta t) = r_2(t) + (\eta + v_m \Delta t)u_2. \quad (3)$$

Provided that the motor complex acts as a harmonic spring $^2,^3$, the force $F_1$ exerted on rod 1 by the motor is given by

$$F_1(t + \Delta t) = k \cdot \Delta x = k \cdot (m_2(t + \Delta t) - m_1(t + \Delta t)), \quad (4)$$

with $k$ being the spring constant. Accordingly the force $F_2$ acting on the second rod is given by $F_2 = -F_1$. In the overdamped case, these forces are balanced by the frictional force $\gamma \cdot v$, whereby $\gamma$ denotes an appropriate friction coefficient. For the velocities of the two polar rods $v_i(t + \Delta t), i = 1, 2$ this yields

$$v_i(t + \Delta t) = \gamma^{-1} F_i(t + \Delta t). \quad (5)$$

Similar expressions can be derived for the angular velocities. The torque exerted by the motor is given

$$M_i(t + \Delta t) = F_i(t + \Delta t) \times (m_i(t + \Delta t) - r_i(t + \Delta t)), \quad (6)$$
with \( \mathbf{m}_i(t + \Delta t) - \mathbf{r}_i(t + \Delta t) \) being the lever arm of the motor. With the overdamped equation of motion, \( \omega_i = \gamma_r^{-1} \mathbf{M}_i \), expressions for \( \omega_{1,2} \) can be derived, whereby \( \gamma_r \) denotes the friction coefficient for rotary motion.

For the relative translational velocity \( \mathbf{v} = \mathbf{v}_2 - \mathbf{v}_1 \) one finally obtains

\[
\mathbf{v} = \frac{2k v_m \Delta t}{\gamma} (\mathbf{u}_2 - \mathbf{u}_1),
\]  

(7)

while the relative angular velocity \( \omega = \omega_2 - \omega_1 \) is given by

\[
\omega = \frac{2k v_m \Delta t}{\gamma_r} \left[ 2v_m \Delta t + \frac{(\mathbf{r}_2 - \mathbf{r}_1) \cdot (\mathbf{u}_1 - \mathbf{u}_2)}{1 - \mathbf{u}_1 \mathbf{u}_2} \right] (\mathbf{u}_2 \times \mathbf{u}_1).
\]  

(8)

At this point it is favourable to compare these expressions to the velocity models derived in \(^4\) starting from generic symmetries. The relative translational velocity \( \mathbf{v} \) corresponds to the \( \beta \)-term in Ref. \(^4\), while the first term in expression (8) is equivalent to the \( \omega_0 \)-term of Ref. \(^4\). The second term of expression (8) was not considered before, but obeys the generic symmetry relations postulated for motor mediated two filament interactions \(^4\).

**Parameters** For all simulations the diffusion constant was adjusted to \( D = 10^{-13} \text{ m}^2/\text{s} \) \(^5\) and the viscosities were set to \( \gamma = 10^{-8} \text{ Ns/m} \) and \( \gamma_r = 2 \cdot 10^{-8} \text{ Ns/m} \) respectively \(^6\). The motor velocity was set to \( v_m = 1 \mu\text{m/s} \) and the filament length corresponds to 10 \( \mu\text{m} \). The off rate of the motor filament amounted to \( r_{\text{off}} = 0.01 \) and the attachment rate of the crosslinker to \( p_{\text{on}} = 0.01 \).

**Limitations of the simulation** With the incorporation of the three basic processes, active transport, crosslinking and forced unbinding, the simula-
Figure S5: Schematic representation of a motor mediated two filament interaction. The two filaments are defined by their length \( L \), the position of their centers of mass \( r_{1,2} \) and the unit vectors of their respective orientation \( u_{1,2} \). The motor complex (red) is located at the intersection of the two filaments. For the initial position \( m \) of the motor the relation \( m = r_1 + \xi u_1 = r_2 + \eta u_2 \) hold, whereby \( \xi \) and \( \eta \) refer to the Starley coordinates of the interacting filament pair.

The simulation can qualitatively reproduce several key results of the experiment. This includes the cluster formation and the coarsening process, the superdiffusive behavior and the dependence of the mean run length on the cluster size.

Despite one should bear the limitations of the approach in mind. The phenomenological description of the forced unbinding kinetics corresponds to a local force balance, as it only models force induced unbinding processes within individual clusters. Long range force percolation that may become important at high actin densities is omitted in this approach. As a consequence the simulation cannot account for possible collective motion patterns.

Further the simulation does not take the polydispersity of the actin fascin structures into account and the individual rods are not able to fragment. This
mainly affects the rupture events within clusters that occur more seldom compared to the experiment.
References

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