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Motor-free force generation in biological systems

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Abstract

A central part of soft matter physics is the investigation of effects in an active environment. These systems are driven out of equilibrium by a constant energy consumption. In biological systems, for instance, energy is consumed in the dynamic polymerization process of cytoskeletal filaments or by motor-filament interactions. These active processes convert chemical energy into mechanical work and impede a trapping of cellular structures in thermodynamically frozen states. Thus, active soft matter is crucial for biological systems to fulfill a broad range of tasks. Inherent physical principles relying on entropy maximizing arguments, however, cannot be easily switched off even in active systems. Cells might even employ these principles to accomplish certain tasks without the need to arrange elaborate, energy dissipating structures. Within the presented studies we demonstrate possibilities how biological relevant forces can be generated in the absence of any active accessory proteins. The presented studies are based on the cytoskeletal key components actin and microtubules. We demonstrate different approaches ranging from light induced softening to cross-linker expansion, which realize entropy driven contractions of the according system.

Keywords: Actin, microtubules, force generation, motor-free, cross-linker

1 Introduction

Cells are the fundamental biological building blocks of living organisms. A central module of cellular structures is the cytoskeleton. It comprises three main components: actin, intermediate filaments, and microtubules [1]. Presented studies focus on the cellular key constituents actin and microtubules, which are monomeric proteins able to polymerize into long filaments and tubes, respectively. In their polymeric form they can be further arranged into networks, bundles, or even networks of bundles. These emerging structures are employed for a variety of cellular tasks ranging from biological force generation [2] to cell motility [3–6] and division [7, 8] as well as force generation on the filament level [1, 9–11]. Besides dynamic processes, cells employ actin and microtubules for static, stabilizing functions [1].

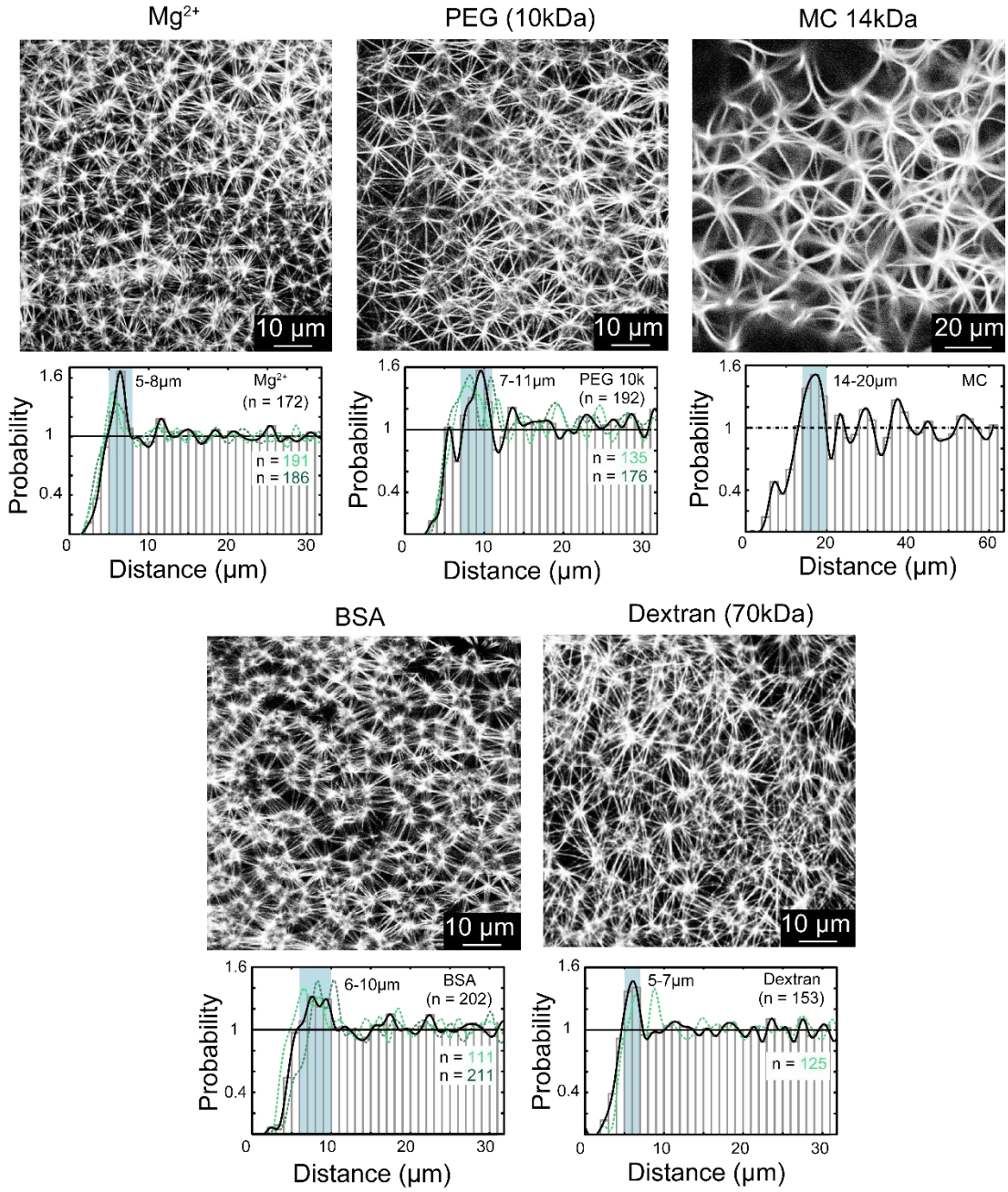

Figure 1: Actin based aster formation without molecular motors.
Aster-like arrangements of actin bundles emerge in the absence of molecular motors or other accessory proteins. Structures are solely formed by magnesium due to counterion condensation or polyethylene glycol, methyl cellulose, the protein albumin, and dextran inducing depletion forces. We were able to show that aster formation relies on an isotropic filament distribution when switching on bundling effects. Motor activity can support this structure formation, but myosin motors are not the inherent basis for this kind of structural arrangement. By detecting center points we derived radial distribution functions for all networks, which display an increased probability for finding a next neighbor in a distance of about 5–10 µm or 14–20 µm, respectively [14]. Adapted from Huber et al. [14].

The ability to employ one key component for seemingly contradictory functions requires accessory proteins, which are able to drastically alter properties of the underlying structure. Cells employ a multitude of additional proteins which are usually either associated to actin or microtubules structures. These proteins can form networks or bundles by cross-linking without the need to consume chemical energy [12]. In addition, active accessory proteins called myosins (interacting with actin) or dyneins and

kinesins (interacting with microtubules) can alter properties of underlying structures by converting chemical energy into mechanical work [13]. These interactions drive a system out of equilibrium avoiding a trapping in thermodynamically stable, frozen states. In that course cells can use identical building blocks for very different tasks.

These influences are described within the frame of active soft matter physics, which has been used to describe a variety of effects such as the formation of asters [15–17]. However, not all effects which are described by motor activity inherently rely on the consumption of chemical energy. Emergence of aster structures, for instance, are conventionally attributed to molecular motors although they can form by self-assembly processes minimizing the system's free energy [14]. We were able to show that aster formation solely relies on an isotropic filament distribution when switching on attracting, bundling effects (Figure 1). In an isotropic system aster formation only relies on any kind of filament attractions, which can be induced by counterion condensation, depletion forces, or cross-linking [14, 17, 18].

In the case of actin based aster formation, myosin motors enhance filament motion and thus anisotropies originating from initial convective mixing or rapid polymerization will decay faster [14]. Thus, an isotropic filament distribution is restored quickly after sample assembly, which is crucial for aster emergence. This example illustrates that inherent physical principles can lead to structure formation based on self-assembly arguments which can be supported by motor activity. However, the supporting role of myosins can be easily misinterpreted in this case covering the inherent basis of aster arrangements.

We present further experimental evidence of inherent physical principles leading to biological force exertions which were conventionally attributed to motor activity. These processes rely on entropy maximizing arguments, which cannot be easily switched off even in active systems. Cells, for instance, might even employ these principles to fulfill certain tasks without the need to arrange elaborate, energy dissipating structures.

2 Entropic contractions of actin structures

Contractile actin structures are a crucial element for biological force generations [19]. These active processes mainly rely on myosin head domains able to bind to actin filaments or bundles. By hydrolyzing ATP to ADP, myosin head domains undergo a conformational change – the so-called power stroke – leading to a force exertion onto the bound actin filaments [20]. In physical terms, this cyclic process converts chemical energy into mechanical work for force generation. Due to these active interactions, actin–myosin structures are considered active soft matter. Mechanical properties of these systems depend on energy conversion [21]. For instance, if ATP is present in an uncross-linked actin network enriched with myosin minifilaments, motor activity enhances filament sliding and the system is fluidized [13]. In the presence of additional cross-linking proteins, myosin motors induce a contractile tension in the actin network leading to a stress hardening of the system [22]. As a result, structures comprising myosin motors, ATP, and actin filaments have been generally considered the minimal systems for actin based contraction [11, 17, 23].

These contractions play a fundamental role in cell motility and facilitate the retraction of the rear of a migrating cell [1]. However, also myosin knock-out cells have been reported to migrate [24]. The

retraction of the rear is crucial for cell motility and thus other mechanisms have to be employed to exert necessary contractile forces.

An alternative concept explaining motor-free contractions involves depolymerization of the cytoskeleton. Depolymerization processes have been proposed to cause contractile forces solely by an entropy gain without the need to convert chemical energy into mechanical work [25]. By severing a filament within a semiflexible network, the surrounding filaments can fluctuate with additional modes (Figure 2). These additional fluctuations decrease the end-to-end distance of a filament and the distance between cross-linkers leading to an overall contraction. This hypothesis has been proven for polymer networks of nematode's major sperm protein and proposed as a potential actin contraction mechanism as well [25].

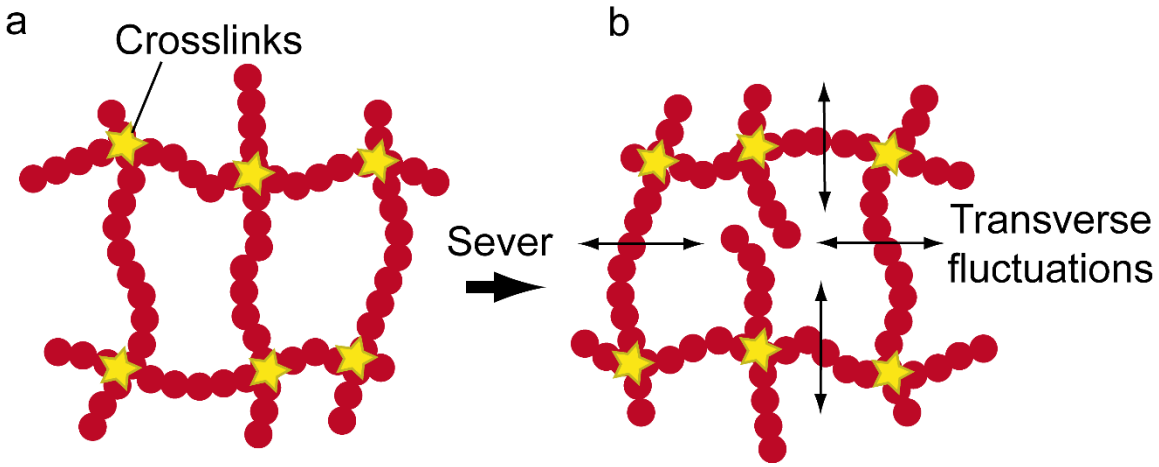

Figure 2: Depolymerization can cause additional fluctuation modes.
(a) Filaments within a cross-linked network show thermal fluctuations which are influenced by fixed inter-filament junctions. (b) Leaving cross-linkers intact and breaking a filament increases the entropic freedom of the polymers. New fluctuation modes are possible decreasing a filament's end-to-end distance. These transverse fluctuations pull cross-linkers closer together and can generate contractile forces. Adapted from Wolgemuth et al. [25].

Inducing depolymerization *in vitro* by actin accessory proteins such as gelsolin or cofilin is experimentally hardly accessible. These proteins do not only severe or disassemble actin filaments, but also alter polymerization dynamics yielding inhomogeneities within a network [26]. Thus, we did not apply any accessory proteins and studied depolymerization effects of actin arrangements with a micro-rheological approach. Fluorescent beads were embedded within actin networks allowing observations as well as manipulation of the system (Figure 3a). By illuminating these beads with the appropriate fluorescence inducing wavelength, free radicals were formed in solution. These radicals reacted with the actin structure and severed filaments leading to a softening of the entire network [27]. Softening and contraction were visualized via embedded beads. For appropriate conditions, free radicals disintegrated exactly the amount of filaments needed to cause contractile behavior while not destroying the entire network (Figure 3b).

However, the appropriate conditions needed to induce these contractions depend on a variety of parameters, which cannot be controlled at the same time. Thus, controlled studies remained elusive and mostly the whole network was disintegrated.

Additionally, these actin networks were cross-linked with heavy meromyosin (HMM), which acts as a passive cross-linker if ATP is depleted within the system. Therefore, the sample was stored for 12 hours at 4 °C allowing actin treadmilling to consume all the ATP in the system. If only a minor amount of ATP would have been present, myosin motors would have been active and networks not entirely cross-linked. In these states, myosin would have fluidized the whole networks impeding controlled contractions [13]. To verify the findings of contractile actin network due to light induced softening further measurements with controllable parameters are needed. To exclude any motor activity, passive cross-linkers such as alpha-actinin or fascin need to be employed. However, more importantly the procedure of light induced softening has to be further characterized to allow controlled manipulations with defined parameters.

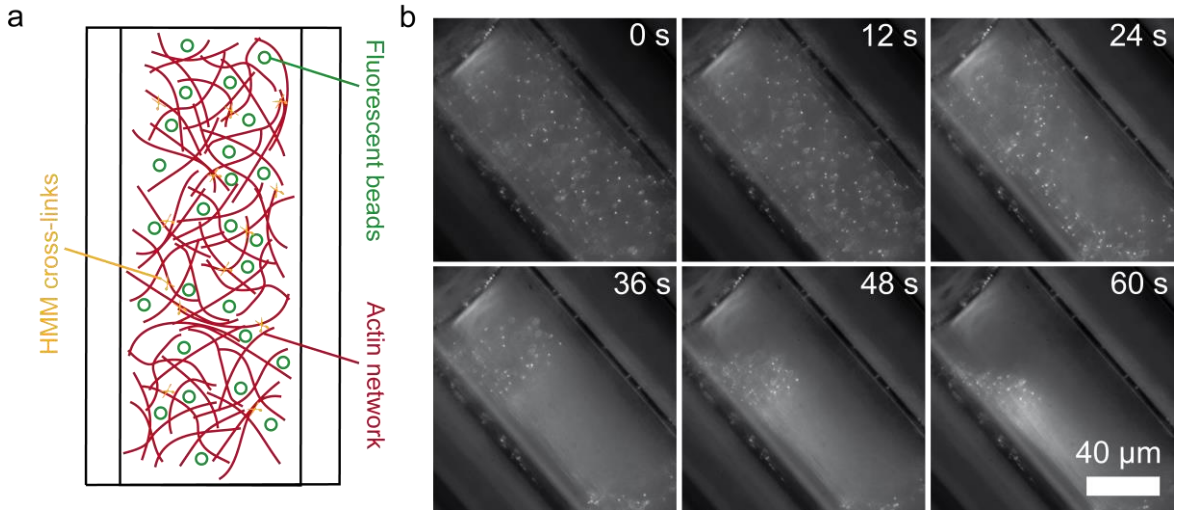

Figure 3: Light induced contraction of an actin network.
(a) A glass capillary (80 µm diameter) is loaded with the sample solution: 1 mg/ml actin labeled and stabilized with rhodamine-phalloidin, cross-linked by HMM (actin:cross-linker 1:160), and enriched with 0.5 µm yellow-green beads. Polymerization of actin is induced directly prior to sample assembly to minimize shear alignment. The system was equilibrated over night at 4 °C to deplete the ATP, which ensures HMM to be inactive acting as a stable cross-linker. Contraction was induced by irradiation with green light causing few actin filaments to depolymerize. (b) Light induced contraction. A one minute time course of the contraction of an actin network is displayed. Fluorescent tracer particles are imaged and illustrate the underlying actin network. Upon illumination beads revealed a significant network contraction.

A first study revealed that fluorescence induced softening cannot be sufficiently suppressed by oxygen scavengers such as DABCO or glucose–glucose oxidase [27]. Quantitative analysis were done with micro-rheological methods which have shown a drastic softening of the entire network (Figure 4).

This softening effect was evaluated for cross-linked and entangled networks interspersed with fluorescent beads by measuring according time evolutions of the MSDs at a lag time $\tau = 2$ s. For cross-linked networks thermal fluctuations increased quickly. Entangled networks displayed a similar trend, but converged towards a slightly higher plateau value for long times (Figure 5) [27].

Obvious differences can be explained by the different architectures of the networks. Cross-linked networks initially confine beads more tightly, whereas beads in entangled networks already showed detectable fluctuations [27]. However, both network types displayed this softening effect and we expect that beads cause not only cross-link breaking but also a disintegration of actin filaments in their surroundings [27].

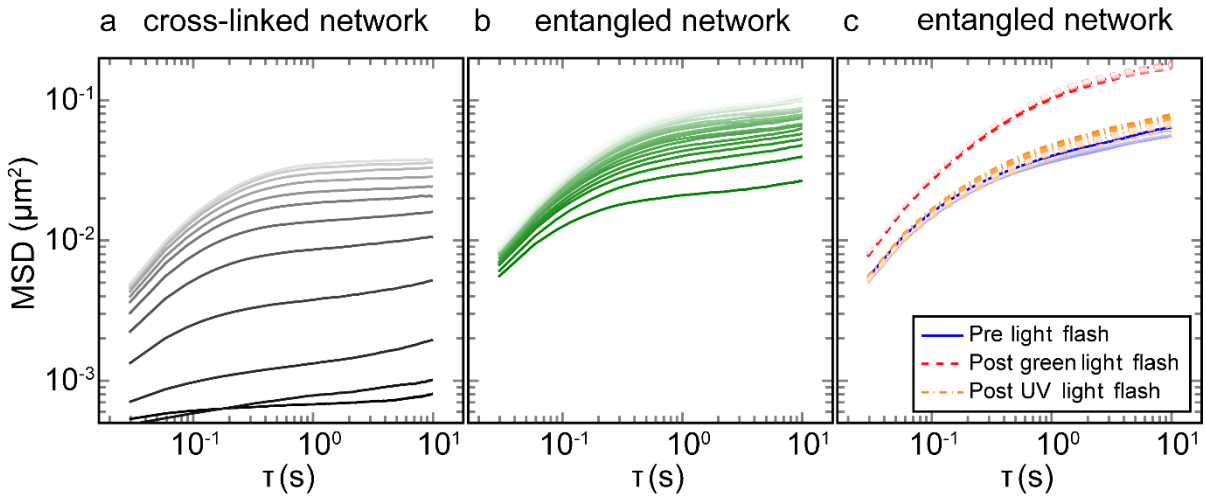

Figure 4: Time dependent responses of actin networks caused by fluorescence.
The time dependent mean squared displacement (MSD) of 1 µm NYO beads in 1 mg/ml actin networks in cross-linked and entangled networks is shown. The MSD was calculated for consecutive 100 s intervals and is plotted against the lag time τ. Consecutive time intervals are displayed as a fade in color. (a) A HMM cross-linked network (1.34 µM HMM) was observed for 1200 s, and (b) an entangled network for 1800 s with fluorescent light. (c) An entangled network was observed for 1900 s with bright field microscopy before and after a 60 s high intensity flash of green and UV lights, respectively. Adapted from Golde et al. [27].

Using micro-rheological methods employing bright field microscopy have shown that whenever fluorescence was induced, free radicals formed and locally destroyed the surrounding network (Figure 5). However, bright field observations did not cause any network disintegration and networks remained virtually unchanged for long time periods [27].

These findings question earlier micro-rheological studies which employed fluorescent beads. Thus, bright field imaging for micro-rheology should be used accepting the lower spatial resolution [27]. However, this study proves that this softening mechanism can be used for active manipulations, but requires well controlled conditions, which could not be realized in the performed bulk measurements.

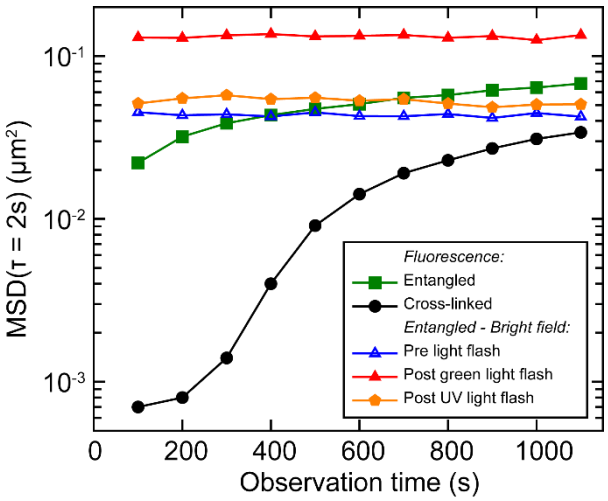

Figure 5: Time dependent responses of actin networks with differing observation methods.
The influence of fluorescence to actin networks (1 mg/ml) was investigated by time dependent MSD's of 1 µm NYO beads with differing methods. The MSD at one distinct lag time $\tau = 2$ s is displayed for the entire observation time t. Picture taken from Golde et al. [27].

In summary, we investigated the possibility to exert biological forces without employing molecular motors. Actin networks were influenced by fluorescent beads, which lead to free radicals within solution when illuminated with the appropriate wavelength. Furthermore, this method allowed manipulations of these actin networks without the need to employ any additional accessory proteins. Additional proteins were intentionally neglected since side effects could not be controlled sufficiently.

Optical manipulations in an appropriate regime indeed yielded contraction events of the network. However, these contractions could be hardly evaluated since the amount of forming free radicals was experimentally inaccessible. Even slight variation of bead diameters and non-uniform bead distributions throughout the sample impeded further controlled studies. The softening effect of actin networks due to fluorescence, however, has proven to be a persistent effect and has to be considered for further studies. Especially studies employing micro-rheology have to take these findings into account and we recommend to use bright field microscopy for these investigations.

Observed network contractions are a first hint that actin structures can exert contractile forces without the need to convert chemical energy into mechanical work by molecular motors. Further studies should employ attracting mechanisms, which are also feasible in cellular systems. Counterion condensation or depletion forces due to molecular crowding might prove to generate forces in a biological relevant regime [1]. These mechanisms are solely based on minimization of the free energy and would not rely on an energy source such as ATP or GTP.

3 Generation of directed forces in microtubule networks by diffusible cross-linkers networks

Diffusion is a crucial transport mechanism in biological matter and originates from random thermal motion of molecules. It can be employed for generating directed forces when involved molecules are spatially confined. A well-known example is a gas spring. Expansions of a gas compressed in a cylinder can be described as an entropic process maximizing the total number of possible microscopic states in the system. Within the presented project, we investigated if analogous descriptions can be applied to subcellular systems. More precisely, cytoskeletal networks have been studied showing that diffusible molecules can indeed generate entropic forces [28]. We note, that this section (generation of directed forces in microtubule networks by diffusible cross-linkers) mainly reviews results recently published in [28] – for further details the reader is referred to this publication.

Molecules in cellular systems are usually not confined within three dimensions. They are rather restricted to two dimensions or even to one dimension. Confinement to one dimension, for instance, has been reported for diffusion of proteins along microtubules [29]. Additionally, the ends of microtubules have been shown to act as diffusion barriers for proteins, which are involved in forcefully tethering kinetochores to the shrinking ends of depolymerizing microtubules [30–32], as well as for diffusible microtubule cross-linkers [33]. A well-known diffusible microtubule cross-linker is S. pombe Ase1 (a member of the Ase1/PRC1/Map65 family), which presumably stabilizes bipolar microtubule arrays. Ase1 localizes at anti-parallel microtubule overlaps in the midzone of the mitotic spindle during anaphase [34] and the interphase microtubule array [35]. Forces generated by associated molecular motors and dynamic microtubules are assumed as the main processes in remodeling both of these bipolar

microtubule structures [36–38]. However, bipolar microtubule arrays are destabilized and disassemble in the absence of Ase1 [34, 35, 39]. Additionally, Ase1 cross-linkers decrease microtubule–microtubule sliding [33, 36] and arising friction forces by microtubule-Ase1 interactions might be required to balance motor activity within networks. Since Ase1 can diffuse in the confined space between microtubule overlaps [33, 40], we report that Ase1, apart from generating friction, can also generate entropic forces [28].

To study forces due to confined Ase1 cross-linkers *in vitro*, we formed overlapping microtubules by immobilizing dimly rhodamine-labeled "template" microtubules on a coverslip. Subsequently, we allowed 50 pM Ase1-GFP to diffusively bind to this template microtubules and flushed in brightly rhodamine-labeled "transport" microtubules afterwards. These transport microtubules were bound to template microtubules by using a solution without Ase1-GFP. This procedure effectively removed free Ase1-GFP molecules from the system (Figure 6). After these free molecules were removed, we applied a hydrodynamic flow of assay buffer without Ase1-GFP to slide transport microtubules along template microtubules. This step generated partial overlaps with reduced overlap lengths (Figure 6a/b). Since diffusible Ase1-GFP molecules show a higher affinity for microtubule overlaps than to single microtubules [33], they did not leave the overlap regions during this process. The reduction of overlap lengths accordingly increased the confinement of the cross-linkers. When the flow stopped, overlap lengths increased by directed sliding of the transport microtubules (Figure 6b). Within this expansion process confined Ase1-GFP molecules redistributed uniformly in the overlap regions by one-dimensional diffusion. We tested that no Ase1-GFP molecules were lost during this procedure, which is illustrated by the constant integrated Ase1-GFP fluorescence intensity along the overlap regions (data not shown). Compression and expansion events were cyclically repeated to resemble the macroscopic mechanism of a gas spring (Figure 6c).

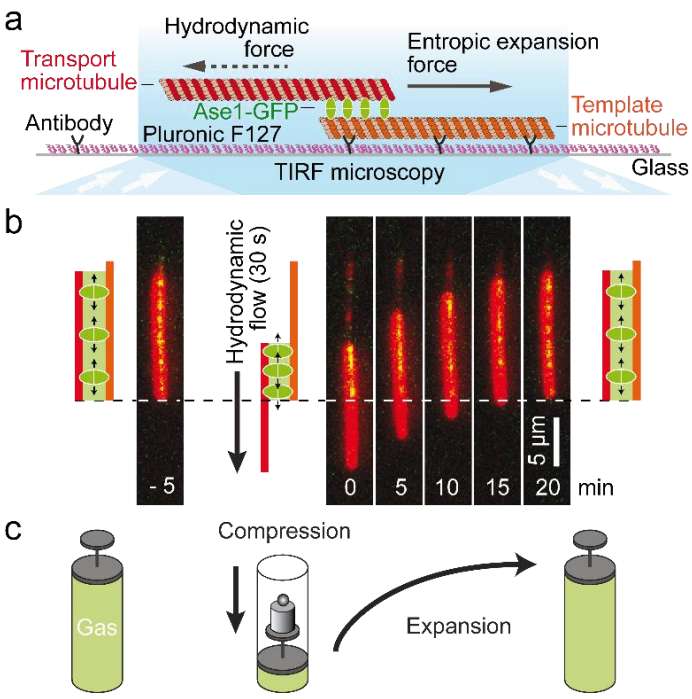

Figure 6: Sliding of partially overlapping microtubules induced by entropic expansion of diffusible cross-linkers.
(a) Experimental scheme of the sliding assay of a transport microtubule (red) along an immobilized microtubule (orange) induced by Ase1.
(b) Fluorescence time-lapse of the sliding motion. Multichannel micrographs of the microtubule overlap display positions of the freely moveable microtubule (red) as a function of time prior and after of flow-induced compression of Ase1-GFP (green). Before imaging freely moving Ase1-GFP was discarded from solution. Schemes show the conformation of microtubules prior and immediately after the application of the hydrodynamic flow and the end of the experiment. The end of the immobile microtubule is shown by the dashed line.
(c) A gas spring describes the analog of the molecular process and expands when the external load is reduced. Adapted from Lansky et al. [28].

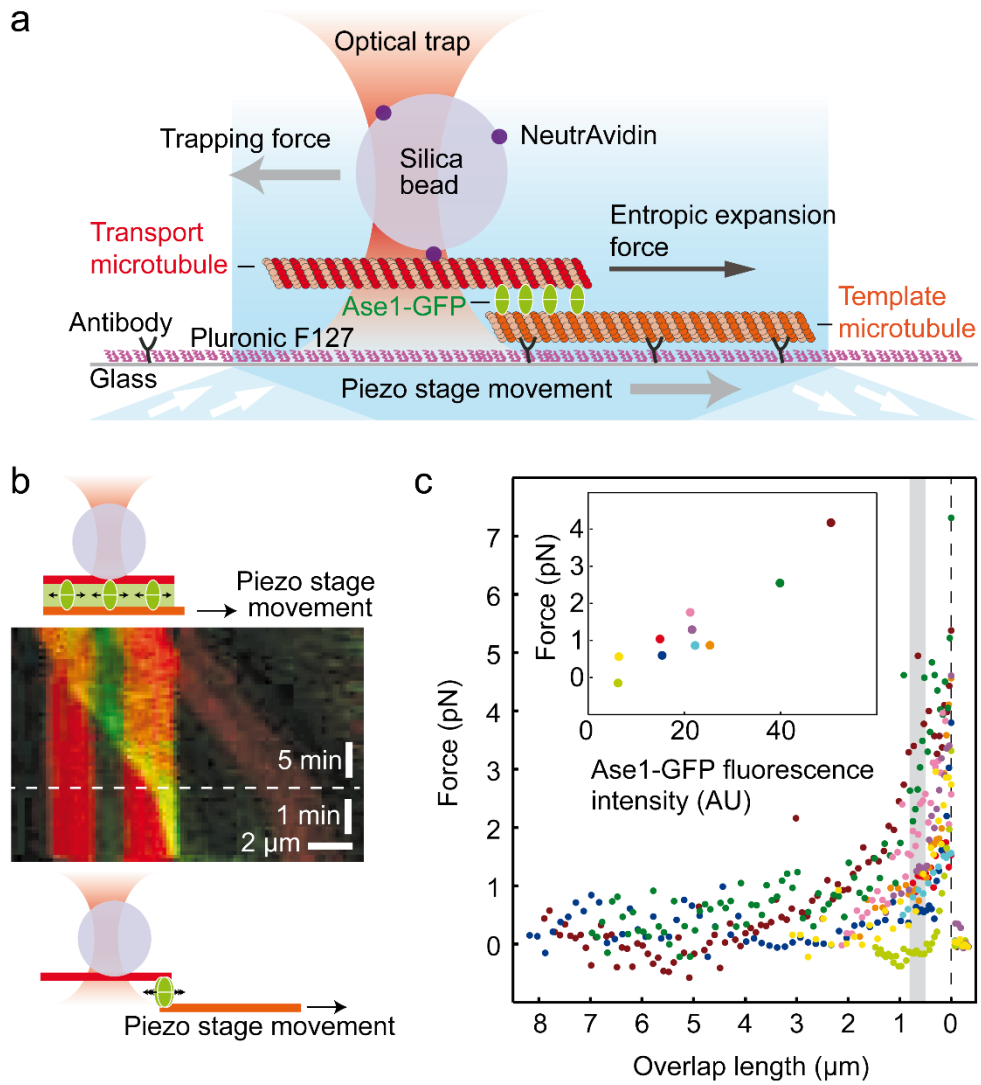

Figure 7: Measuring forces exerted by confined Ase1-GFP molecules in microtubule overlap regions.
(a) Measurements were carried out using optical tweezers. A NeutrAvidin-coated silica bead was trapped and attached to a free, biotinylated microtubule (red). The fixed microtubule (orange) was moved by a piezo translational stage. The center of the trap with the free microtubule was held at a constant position yielding a sliding of microtubules relative to each other. (b) These experiments can be visualized by multichannel kymographs showing the sliding of the weakly labeled, fixed microtubules (movement driven by piezo stage) relative to the glowingly labeled, optically trapped microtubule. When the microtubules overlap shortened, the Ase1-GFP density increased. The movement of the piezo stage was slowed down roughly two minutes before the microtubules started to separate to obtain more important data points. See also Lansky et al. for snapshots of the event as well as a representative event in which the optical trap was deactivated before the microtubules separated resulting in the expansion of the overlap. (c) Shown are equilibrium bead displacements which correspond to steady state forces caused by Ase1-GFP confined in the overlaps. Forces are plotted as a function of overlap lengths. Shown are ten independent experiments shown in different colors. Initial fluorescence intensities in the overlap region correspond to 15 to 80 Ase1-GFP molecules. (Inset) Forces are displayed in a relation to the fluorescence intensity in the overlap. Data is averaged for overlaps of lengths ranging from 0.6 to 0.8 µm (corresponds to the grey box in the main panel; the color-coding is consistent for according experiments). Overlap lengths and forces were offset-corrected by assuming that the overlap length is zero right before the microtubules were pulled apart (dashed line) and that the force is zero after the microtubules were pulled apart. Adapted from Lansky et al. [28].

We quantified arising forces, which were solely exerted by Ase1 confinement between partially overlapping microtubules, by optical tweezers (Figure 7). In a first step we formed microtubule overlaps in a similar manner as described above. Subsequently, we attached a silica micro-sphere to a transport microtubule by optical tweezers in the absence of Ase1-GFP in solution [28]. After

establishing this conformation, we used a piezo translation stage and moved the template micro-tubule in steps relative to the laser trap in the direction along the longitudinal axis of the template microtubule. In that course we established partial microtubule-overlaps and compacted Ase1-GFP until the microtubules were pulled apart (Figure 7a/b). After every step, the system was allowed to equilibrate before measuring the force. These forces have been found to increase with a decreasing overlap length reaching values up to 3.7 ± 1.8 pN (average $\pm$ SD, n = 10) just before the two microtubules were pulled apart (Figure 7c) [28]. Additionally, fluorescence intensities measurements revealed that forces increased linearly with increasing Ase1-GFP densities in the overlap regions (Figure 7c, inset) [28].

To explain the origin of observed forces generated by Ase1, we analytically modeled the mutually exclusive binding of cross-linkers to discrete binding sites along a single protofilament in a microtubule overlap (Figure 8) [28]. In brief, for a constant number of confined cross-linkers in the overlap – when no cross-linkers bind into or unbind from the overlap (scenario as in Figure 6 and Figure 7) – the entropic expansion force F acting on the transport microtubule is found to be given by the one-dimensional analog of the ideal gas law $FL \cong nk_{\mathrm{B}}T$ [28], with L being the overlap length, n the number of cross-linkers within the overlap, k_{B} the Boltzmann constant, and T the absolute temperature. This model is consistent with our experimental results showing that forces increase linearly with the density of the cross-linkers in the overlap (Figure 7c, inset). Quantitative tests of the relation of force and cross-linker density is not feasible due to experimental uncertainties in overlap lengths and the amount of proteins. However, the range of maximum measured forces is predicted

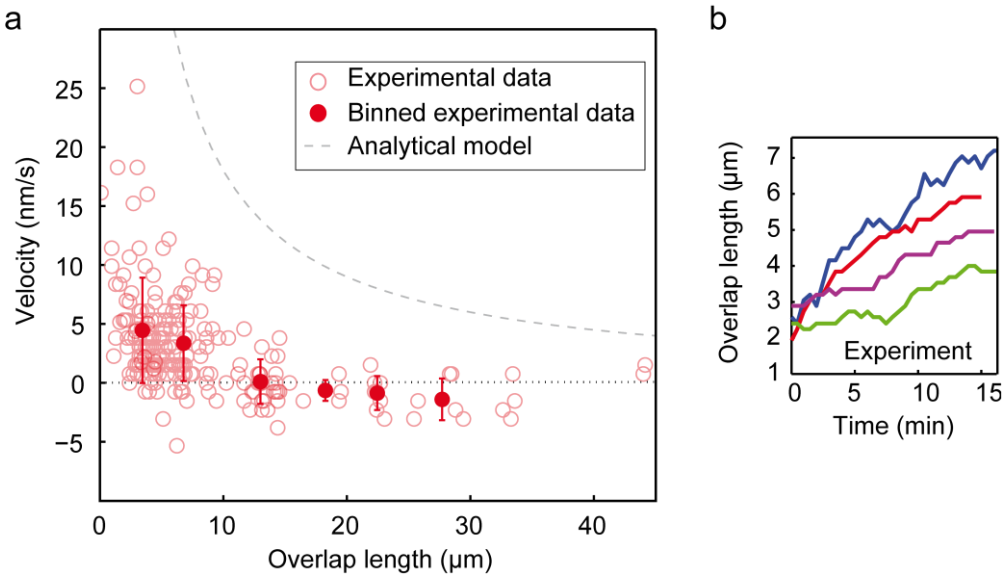

Figure 8: Ase1-GFP entropy gain combined with an exponential scaling of friction forces can explain the expansion of microtubule overlaps in the absence of Ase1-GFP.
(a) Averaged velocities of Ase1-GFP induced microtubule sliding are given as a function of overlap length. Presented are experimental data (red open circles, 95 events, 48 microtubules, experiments as presented in Figure 6b and results from the analytical model (grey dashed line, $v_{\mathrm{MT}} = 2D_{\mathrm{Ase1}}^{\mathrm{MT}}/L$ with $D_{\mathrm{Ase1}}^{\mathrm{MT}} = 0.085 \pm 0.007$ µm^2 s^{-1} assuming a constant number of Ase1-GFP in the overlap). Overlaps were allowed to expand for a minimum of 15 minutes. Binned averages ($\pm$ SD) of experimental data shown as solid red circles. (b) Time traces of overlap expansions acquired from the experiments (data as shown in Figure 8a) are given. Different color-coding illustrate individual events. Visible variability of the time traces reflects the stochastic nature of the underlying force generating mechanism. Adapted from Lansky et al. [28].

correctly. The model predicts the generation of forces in the range of 1 pN if cross-linkers are maximally compressed between two microtubule protofilaments. In this case, all binding sites within the overlap region are fully occupied by Ase1 molecules. Structural work on Ase1 homologs suggests that the necessary high cross-linker densities are indeed possible [41]. Observed maximal forces of 3.7 ± 1.8 pN potentially indicate that multiple rows of Ase1 cross-linkers bind to neighboring protofilaments in the overlap [28].

In addition to these findings, we tested if the combination of these entropic forces and frictional drag exerted by the Ase1 cross-linkers can also elucidate the observed sliding velocities of transport microtubules in absence of external load (scenario as in Figure 6). Viscous drag exerted by the solution was neglected due to its small contribution at low velocities [42, 43]. We described the frictional drag coefficient γ of a single Ase1-microtubule link following the Einstein relation $\gamma = k_{\mathrm{B}}T/D_{\mathrm{Ase1}}^{\mathrm{MT}}$ [44], where $D_{\mathrm{Ase1}}^{\mathrm{MT}}$ is the diffusion constant of a single Ase1 molecule on a single microtubule [28]. The velocity of the overlap expansion is given by $v_{\mathrm{MT}} = 2D_{\mathrm{Ase1}}^{\mathrm{MT}}/L$ when assuming a linear dependence of the frictional drag on the number of diffusible cross-linkers [28, 43]. This expression is independent of the number of cross-linkers in the overlap and qualitatively reproduces the measured velocities (Figure 8b) [28].

These entropic forces might be even sufficient to counteract forces generated by microtubule-cross-linking motor proteins. To test this hypothesis we formed and imaged microtubule overlaps in the presence of Ase1-GFP and D. melanogaster kinesin-14 Ncd (Figure 9). Ncd does not directly interact with Ase1 [33] and started to slide the microtubules apart. Within this process, the Ase1 molecules were compressed in the shortening microtubule overlaps. The number of bound Ase1-GFP linkers stayed roughly constant during this compression due to their high affinity to the overlap region. Additionally, the number of Ncd molecules decreased linearly with decreasing overlap lengths [33]. Typically after 10 minutes the sliding motion stopped and lengths of the overlaps remained constant. This result emphasizes that sliding forces induced by motor activity can be balanced by the entropic expansion force of Ase1. The description is analogous to a gas spring, where external loads are balanced by the internal pressure of the gas [28].

In line with the hypothesis that the Ncd sliding forces are balanced by the Ase1 generated forces, we found that the overlap lengths immediately increased when either (1) Ncd motors were deactivated by exchanging ATP for ADP in the assay buffer (Figure 9a) or (2) cross-linkers were added into the overlaps by increasing the Ase1-GFP concentration in solution (Figure 9b) [28]. Thus, analog to a gas spring the overlap expanded when the force balance was tipped by either (1) reducing the opposing, external load or (2) raising the internal pressure by increasing the number of molecules in the overlap. These findings demonstrate that diffusible cross-linkers are capable of generating entropic expansion forces of the same order of magnitude as the forces generated by multiple molecular motors [28].

Our findings show that the mechanism of entropic force generation due to confined molecules is a universal phenomenon and has to be considered beyond the Ase1/PRC1/ MAP65 family of microtubule cross-linking proteins. Nucleosome unwrapping, for instance, was recently quantitatively explained by a one-dimensional pressure generated by DNA binding proteins diffusing along a DNA strand [45].

Moreover, entropic forces can be also generated in 2D systems. This is illustrated by the example of crowding of membrane-bound proteins generating a lateral pressure leading to bent membranes [46].

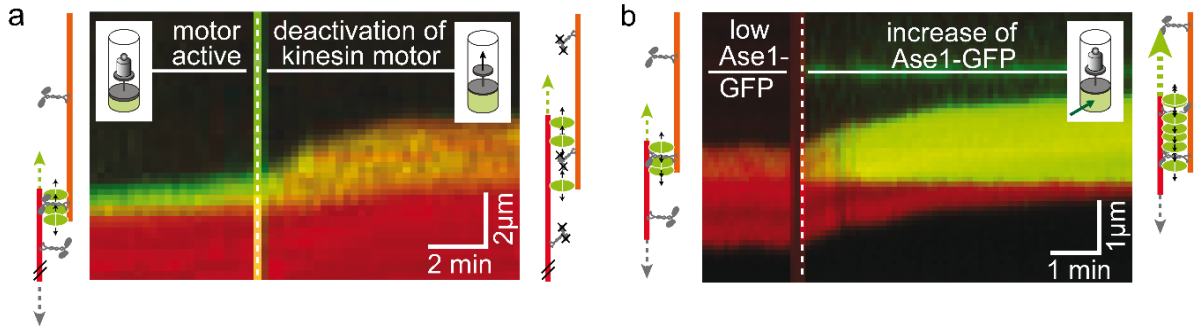

Figure 9: Entropic forces caused by Ase1 balance motor activity of multiple Ncd motors.
An overlay of multichannel kymographs shows the sliding of a transport microtubule (red channel) on top of an immobilized microtubule (very dimly labeled). Partial microtubule overlaps were formed in the presence of Ase1-GFP and Ncd. The contributing forces established a balance between Ncd-motor generated forces (acting in the direction of decreasing overlap length) and Ase1-GFP entropic forces (acting in the direction of increasing overlap length). (a) Shown is the shift of the force balance after deactivation of the Ncd-motors by exchanging ATP with ADP in the assay buffer. Due to entropic expansion of Ase1-GFP molecules bound in the overlap regions, the free microtubule slid towards an increasing overlap length. The Ncd-motor concentration was kept constant (300 pM in solution) during the expansion phase. Additionally, no free Ase1-GFP was present in the solution. (b) Shifting the force balance due to an increased Ase1-GFP concentration in solution (from 91 pM to 1400 pM) lead to an increased binding of Ase1-GFP molecules in the overlap region. The free microtubule slid towards an increasing overlap length and against the ATP-driven active force of Ncd (kept at a constant concentration of 300 pM in solution). Adapted from Lansky et al. [28].

In the cytoskeletal system, the constriction of the actin contractile ring has been conventionally attributed to a non-muscle myosin II (NMII) translocation of actin filaments. Recent experiments, however, emphasize that NMII motor activity is not required to translocate actin but to cross-link actin filaments [47]. Our findings suggest that NMII is potentially able to generate tensile forces between actin filaments via entropic expansion if these molecules can diffuse between filaments [28].

The presented *in vitro* system allows well-controlled experimental studies of the interplay between entropic-expansion forces, cross-linker-condensation forces, and cross-linker-frictional forces driving the sliding of filaments relative to each other. By examining a minimal system consisting of cross-linkers and microtubules, outside of the cytoplasm, we gained access to biophysical properties of the system that are impossible to access *in vivo* where they are obscured by numerous interdependent processes. Our results demonstrate that the thermal motion of confined cross-linkers constitutes a force-producing element within self-organizing filamentous networks and can complement forces generated by molecular motors and filament dynamics [28].

4 Conclusion

Conventionally, most biological force generating mechanisms are described by energy dissipating processes. These approaches are reasonable since cells are active entities consuming a lot of chemical energy to avoid trapping in thermodynamically frozen states. However, we show that some functions or even structural assemblies can be achieved without employing elaborate, energy dissipating structures. We present a variety of studies and experimental methods suitable to explain biological force exertion based on self-assembly, free energy minimizing arguments. Within these investigations, the cytoskeletal

key components actin and microtubules were used emphasizing the biological relevance of the presented findings. Actin based contractions were initialized by depolymerization forces due to light induced softening. This softening effect, however, can be hardly controlled and eventually lead to disintegration of the entire network. However, some experiments revealed contraction events indicating the possibility of actin based force generation due to depolymerization effects. Further experiments are needed to study the potential role of a controlled structural disassembly. Additionally, further investigations are needed to explore entropic effects in active environments such as cells. The presented force generation due to entropic crosslinker expansion between microtubules is a major step towards understanding the role of entropically driven processes in cells. Exerted forces are on the same scale as forces induced by active microtubules – motor interactions and thus in a biological relevant regime. Presented self-assembly interactions, however, do not rely on converting chemical energy into mechanical work and can even counteract motor activity. This effects can feasibly contribute to cellular processes such as the formation of the mitotic spindle. These findings demonstrate how passive processes can contribute to cellular functions and that self-assembly processes should not be neglected in active systems such as cells.

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