Cross-linker unbinding and self-similarity in bundled cytoskeletal networks

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The macromechanical properties of purely bundled in vitro actin networks are not only determined by the micromechanical properties of individual bundles but also by molecular unbinding events of the actin binding protein (ABP) fascin. Under high mechanical load the network elasticity depends on the forced unbinding of individual ABPs in a rate dependent manner. Cross-linker unbinding in combination with the structural self-similarity of the network enables the introduction of a concentration/time superposition principle - broadening the mechanically accessible frequency range over 8 orders of magnitude.

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unbinding of interconnecting fascin molecules even before non-linear behavior can be evoked.

On the other hand the same effect can be achieved by applying different strain rates to a bundle network with fixed microstructure \( R = 0.1 \) as shown in Fig. 1b. Varying the strain rate by two orders of magnitude leads to a continuous change in the degree of strain-hardening. This underlines the non-universal behavior of the non-linear response of semi-flexible polymer networks as also reported for purely entangled actin solutions \[15\] and isotropically cross-linked networks \[3\]. When the bundle network is sheared with very low strain rates, detaching of fascin molecules is very likely on the timescale of the experiment \( \approx 100 \text{ s} \) in the case of \( d\gamma/dt = 0.125 \text{ % s}^{-1} \). Therefore, the remaining number of connection points between fascin bundles might become too low to evoke strain hardening as in the case of higher shear rates. This result suggests that the elastic response of purely bundled actin networks might be equally determined by the cross-linker concentration and the chosen time scale of the experiment. The non-static nature of actin/fascin bonds introduces the cross-linker off-rate as an important time scale. For a single molecular bond a linear increase of the maximal loading force with the logarithm of the loading rate, \( f_{\text{max}} \sim \ln(d\gamma/dt) \), would be expected \[20, 21\].

To this end we conducted shear rate experiments in such a way, that the non-linear response was completely tuned from strain-hardening to weakening. To further investigate the dependence of \( f_{\text{max}} \) on the loading rate, a weakly bundled network is chosen where the density of bundle interconnection points is low \( R = 0.05 \). For this network type strain-hardening is observed for all strain rates applied (FIG. 2). The maximum strain the bundle network can endure without being reversibly damaged is independent from the strain rate, \( \gamma_{\text{max}} \approx (22 \pm 1) \% \). The maximum stress \( \sigma_{\text{max}} = \sigma(\gamma_{\text{max}}) \) the bundle network can withstand depends logarithmically on the strain rate \( d\gamma/dt \) as depicted in the inset of FIG. 2. The maximum force a single fascin molecule connecting two bundles can hold is given by \( f_{\text{max}} \sim \sigma_{\text{max}} l_c^2 \) whereas \( l_c \) denotes the average distance of two neighboring bundle interconnection points being fixed for a given \( R \). For the data presented here the proportionality \( df/dt \sim d\gamma/dt \) holds. Therefore the Bell-prediction is reproduced surprisingly well - indicating that forced unbinding of actin/fascin bonds gives rise to the observed strain rate dependence. As the macroscopic deformation is transmitted in a non-affine way to the bundle crosslinking points \[6, 22\], not all interconnecting fascin molecules will be loaded with the same force during a strain rate experiment. Surprisingly, the Bell prediction is still fulfilled.

Unbinding of fascin molecules between single bundles should also be observable in the low frequency behavior of the viscoelastic moduli. Indeed, a pronounced minimum in \( G''(\omega) \) can be found in the linear response of such networks. From the frequency spectra we roughly estimate the time scale for these events to be on the order of several seconds which in general matches the typical off-rates measured for various actin-binding proteins \[23, 24\]. The measured frequency spectra of actin/fascin networks show two subpopulations in dependence on \( R \): Interconnected bundle networks exhibit a plateau-like storage modulus \( G'(\omega) \) while the loss modulus \( G''(\omega) \) reveals a well-defined minimum which shifts to higher frequencies with increasing fascin concentration. Networks below the bundling transition (low \( R \)) however, feature frequency spectra that do not exhibit a minimum in \( G''(\omega) \) but nevertheless highly resemble each other.

To obtain appropriate parameters for a putative generalization process an impartial criterion is needed. Therefore for each frequency spectrum the minimum position
in the loss modulus \( \omega^*(G''(\omega)) \) together with the corresponding value of the storage modulus is determined. Normalizing the frequency spectra by these values results in a master curve which shows two different regimes corresponding to distinct network types. Therefore, the master curve is discussed best in two parts: Firstly, at low rescaled frequencies the master curve corresponds to networks in the purely bundled phase (FIG. 3a, rescaled \( G'\omega \) shifted up for clarity). This regime contains a pronounced plateau region in the storage modulus \( G'(\omega) \) accompanied by a clear minimum in the loss modulus \( G''(\omega) \). Secondly, networks before the bundling threshold also show a common frequency dependence similar to a purely entangled actin solution (FIG. 3b).

For an actin concentration of \( c_a = 9.5 \) µM the threshold ratio \( R^* \) was determined to be \( R^* \approx 0.01 \) [4]. Indeed, the scaling parameters obtained for the master curve construction follow a conjoint power law \( G'^{\prime*} \sim (\omega^*)^{-x} \), \( G''^{*} \sim (\omega^*)^{-x} \) in the purely bundled phase, while dropping off this relation for values \( R < R^* \) (see inset of FIG. 3b). For the pure bundle phase a linear relation between the scaling parameters, \( x = 1 \), is obtained. This is an indication of self-similarity as also observed in other soft matter systems [26]. Fascin networks with \( R < R^* \) can also be rescaled with a common power law between the scaling parameters, however \( x \approx 1/2 \). As in this regime the tube model can be applied to describe the mechanical response [4], the addition of few fascin molecules seems to only slightly modify the network properties which does not give a self-similar structure.

The corresponding part of the master curve, \( \omega/\omega^* > 10^3 \), shows a regime \( \sim \omega^{0.5} \) in both viscoelastic moduli right before the crossing-over, \( \omega_{\text{cross}} \sim 1/\tau_e \), where \( \tau_e \) denotes the entanglement time. While the molecular origin of this behavior is still unclear, it is expected for cross-linked semi-flexible polymer networks in an intermediate frequency regime resulting from tensions in the network [4]. It is also reported for entangled actin networks from two-point micro-rheological experiments [26] and interpreted to be due to the diffusive dissipation of long-wavelength longitudinal fluctuations. Only at very short time scales \( t << \tau_e \), and therefore very high frequencies beyond the crossing-over of \( G'(\omega) \) and \( G''(\omega) \), i.e., around 10 kHz, a scaling \( \sim \omega^{0.75} \) due to undisturbed relaxations along single filaments would be expected [4, 27, 28]. Rescaling the loss factor \( \tan(\delta) = G''(\omega)/G'(\omega) \) (supplementary information) shows unambiguously that the \( \omega^{0.5} \) regime observed for actin/fascin networks is indeed an analytical power law as the Kramers-Kronig relations are fulfilled. Possibly, the frequency behavior at intermediate times might be strongly dependent on the network architecture as a regime \( \sim \omega^{0.75} \) has been described for actin networks in a composite phase, as obtained by the cross-linker scruin [29].

Thus the mesoscopic network structure does not only determine the plateau modulus but also the frequency behavior of semi-flexible polymer networks. Despite the fact that for composite networks a master curve description was reported [26], this is not a generic feature for cross-linked actin networks. Purely isotropically cross-linked networks [4] do not allow a master curve construction based on a concentration/time superposition. There, the network elasticity is enhanced by simply decreasing the cross-linker distance \( l_c \) - without any overall change in the network structure. Thus self-similarity does not apply for isotropically cross-linked networks. The master curve construction applied here is only possible if one intrinsic parameter determines structure and mechanical behavior at the same time, which is the case for purely bundled actin/fascin networks. Raising the fascin concentration \( R \) "magnifies" all system properties including the mechanical parameters of its constituting bundles [4, 13]. This creates a coarsening, self-similar network that can be described by the principle of cross-linker concentration/time superposition introduced here.

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**FIG. 3:** Master curve for actin/fascin networks a) in the bundled phase \( R = 0.50, 0.21, 0.05, 0.02, 0.01 \) and b) before the bundling transition \( R = 0.01, 0.005, 0.002, 0.001, 0 \) as described in the text. Closed symbols denote \( G' \), open symbols denote \( G'' \). The inset shows the dependence of \( G'^{\prime*} \) and \( G''^{*} \) on \( \omega^* \) (full line: \( \sim (\omega^*)^{1} \), dotted line: \( \sim (\omega^*)^{1/2} \).
Still, thermal and enforced unbinding of interconnecting fascin molecules could lead to subtle changes in the network structure. The increase of $G''(\omega)$ at frequencies lower than $\omega^*$ is quite weak, which stands in marked contrast to rigor HMM-networks where a steep uprise of $G''(\omega)$ below the minimum position is reported. This difference is attributable to the fact that the mechanical response of an isotropically cross-linked network is dominated by the number of HMM-molecules bound, corresponding to $l_\ast$. Thus unbinding results in a structural and mechanical transition from a cross-linked network to an entangled solution. In contrast, for a purely bundled network the linear response is dominated by the bundle properties and the non-affine deformation mode. Only at large time scales $t >> 1/\omega^*$ it will be slightly affected by the cross-linking degree.

In summary, the binding kinetics of the cross-linking molecules and the network structure determine the network elasticity at high deformations. Forced unbinding of cross-links under mechanical load can tune the non-affine response on intermediate times. Moreover, the coarsening, self-similar structure of purely bundled actin/fascin networks allows a generalization of their frequency behavior. The concentration/time superposition introduced here broadens the measurable frequency window over 8 orders of magnitude simply by altering the typical network length scales tuning $R$ - fully equivalent to the principle of temperature/time superposition used for classical flexible polymer networks. Thus, the chosen time scale crucially affects the mechanical network response as distinct length scales dominate the viscoelastic behavior. As a consequence a generic broad distribution of relaxation times has to be considered describing the frequency behavior of cross-linked semi-flexible polymer networks and living cells [30, 31]. The self-similar in vitro system presented here provides a benchmark to address the challenging question of how single filament and bundle properties, network structure and binding kinetics of ABPs interplay to provide the high adaptability, which is known for the cytoskeleton of living cells. Therefore, the described effects may be more generic for an understanding of adaptable biomaterials.

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FIG. 4: Generalized loss factor curve as described in the text. The unshifted curves are depicted in the inset ($R = 0, 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5$).

From a single macrorheological experiment it is difficult to extract the crossing-over time $\tau_e$ with a decent accuracy. The master curve presented in this work would in principle offer enough reliable data points - albeit normalizing both moduli to the same absolute value cancels out the desired information. In fact, shifting up again the rescaled storage modulus might give the wrong impression that both moduli do not cross over at all in the frequency range probed. Therefore, it is helpful to calculate the loss factor $\tan(\delta) = G''(\omega)/G'(\omega)$. The shape of the loss factor curves drastically changes at $R^* = 0.01$ (see inset of supplementary FIG. 4). This again is in agreement with the bundling transition reported before.

Moreover, these curves can also be generalized (FIG. 4) requiring only one single rescaling parameter, namely the same $\omega^*$ as used for the master curves shown in FIG. 3. For a rescaled frequency range of $10^3 \sim 10^4$ the loss factor master curve exhibits a plateau. In this regime the viscoelastic moduli show the same frequency dependence and have comparable absolute values. Thus the Kramers-Kronig relations are fulfilled implying an analytical power law behavior in an intermediate asymptotic regime. For even higher rescaled frequencies the loss factor finally reaches values larger than 1, indicating that a crossing-over to a viscous dominated regime, $\omega > 1/\tau_e$, does exist but is not sufficiently accessible with macrorheological methods - even not by the application of the cross-linker/time superposition.