Supplementary Material (ESI) for Soft Matter
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Supplementary information for Lieleg et al.: Structural Polymorphism in Heterogeneous Cytoskeletal Networks

Local network elasticity of composite bundle networks

The local network elasticity of an actin/α-actinin network in the composite bundle phase (c_a = 9.5 μM and R = 0.02) is determined by active magnetic tweezer microrheology at 21 °C. After sample cooling and equilibration for several hours the same network is microrheologically probed at 12 °C. The distribution of the microscopic storage modulus at a given frequency, G' (0.1 Hz), is determined by analyzing 80 different bead positions at both temperatures (Fig. 1). A decrease in temperature results in a significant increase of the average value of the distribution, $< G' (0.1\ \text{Hz}) >$. However, the relative distribution width, $\sigma_{rel} = \sigma / < G' (0.1\ \text{Hz}) > \approx 0.22$, is unaffected by the temperature change. This relative distribution width characterizes the degree of micromechanical heterogeneity and resembles the value obtained for isotropically cross-linked networks [1].

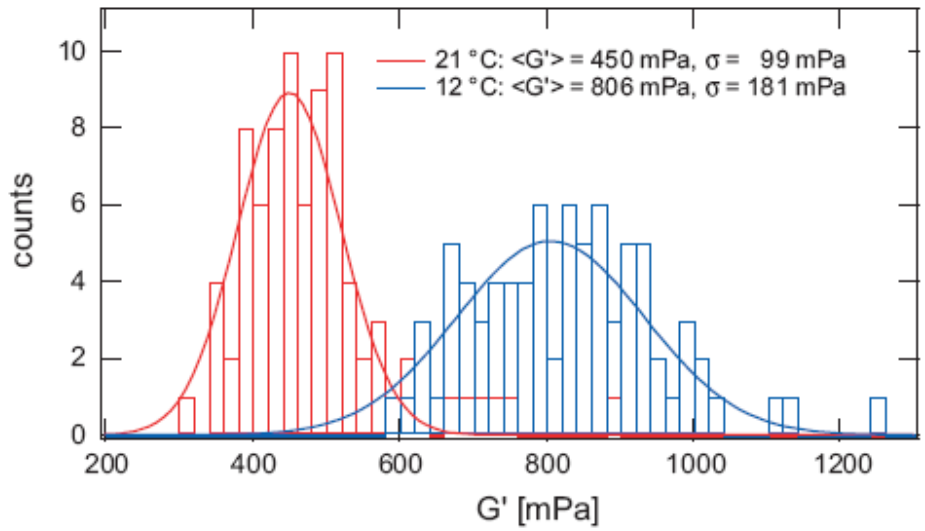

FIG. S1: An α-actinin/actin network (c_a = 9.5 μM and R = 0.02) is investigated by microrheology. The distribution of the microscopic storage modulus at a given frequency, G' (0.1 Hz), is shown for 21 °C (red) as well as 12 °C (blue). A Gaussian is fitted to the distribution data to determine the average value $< G' >$ and the distribution width σ.

[1] Y. Luan, O. Lieleg, B. Wagner, and A. Bausch, Biophys. J. **94**, 688693 (2008).

Robustness of the simulation results

As discussed in the manuscript, finite element simulations are employed to study the dependence of the network stiffness on the degree of heterogeneity. In order to assure the independence of these results from the assumptions made on the network microstructure, the simulation results shown in the manuscript are compared to results that are obtained for a different set of geometrical parameters. As depicted in Fig. S2A, the simulation results are largely insensitive to the cluster size, i.e. the radius of the sphere into which the beams are rearranged when the cluster is formed. Similar results are also obtained for varying cross-link distances (Fig. S2B), i.e. the critical distance which is required to introduce a cross-link between two nodes of neighbouring beams.

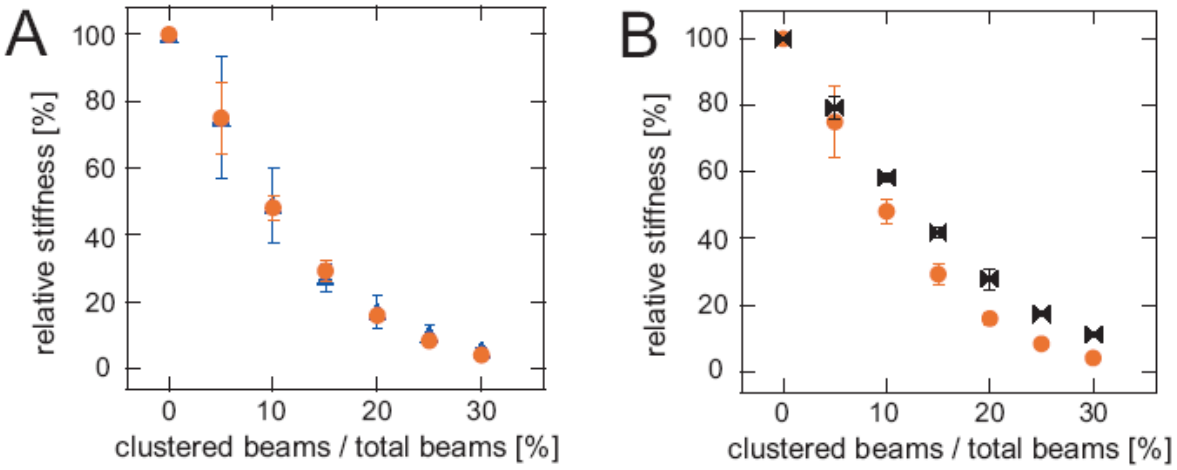

FIG. S2: Finite element simulations of cross-linked bundle networks with increasing degree of heterogeneity: The relative network stiffness is shown as a function of the degree of heterogeneity for different cluster sizes (A) (circles: 5 μm cluster radius, upward triangles: 50 μm cluster radius) as well as different critical cross-link distances (B) (circles: 500 nm cross-link distance, butterflies: 625 nm cross-link distance).

Negligible hysteresis of composite bundle networks

An actin/*α*-actinin network (*R* = 0.02) is polymerized at 12 °C. An intermediate heating step to 21 °C followed by a cooling step back to the initial temperature results in a plateau modulus *G'* (10 mHz) which is only slightly higher than the initial value (Fig. S3A, *G'*(*f*) and *G'*(*f*) are shown in Fig. S3B).

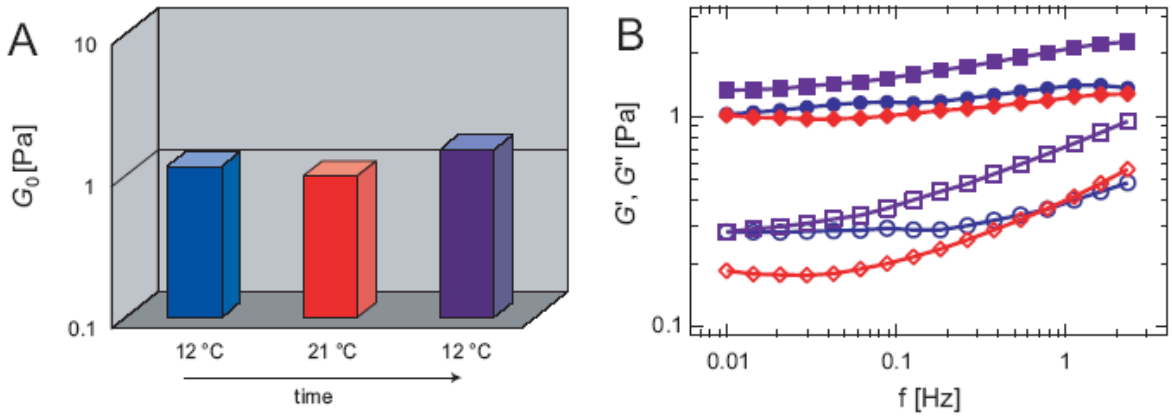

FIG. S3: (A) Plateau modulus G_0 of composite *α*-actinin bundle networks (*R* = 0.02) at different temperatures. After polymerization at 12 °C (blue) a heating step up to 21 °C (red) followed by re-cooling to 12 °C (purple) was applied. (B) Frequency spectra of the *R* = 0.02 network shown in the front line of (A) directly after polymerisation at 12 °C (blue circles), after heating to 21 °C (red diamonds) and after re-cooling to 12 °C (purple squares). Closed symbols denote *G'(f)*, open symbols denote *G''(f)*.

Thermal stability of bundle clusters

In order to investigate the stability of actin/*α*-actinin clusters towards temperature, an actin/*α*-actinin bundle cluster network (c_a = 4.25 μM and R = 0.5) is polymerized at 10 °C and observed with confocal microscopy. A typical cluster occuring in such networks is shown in Fig. S4A. After 60 min of polymerization, the network is heated up to 30 °C and equilibrated for 30 minutes. To compensate for drift, a comparable confocal layer in the bundle cluster is chosen after this heating procedure. As shown in Fig. S4B the heterogeneous network structure is stable despite the drastic temperature change, only minimal changes in the network configuration occur. Note, that bleaching effects might have changed the absolute fluorescence intensities.

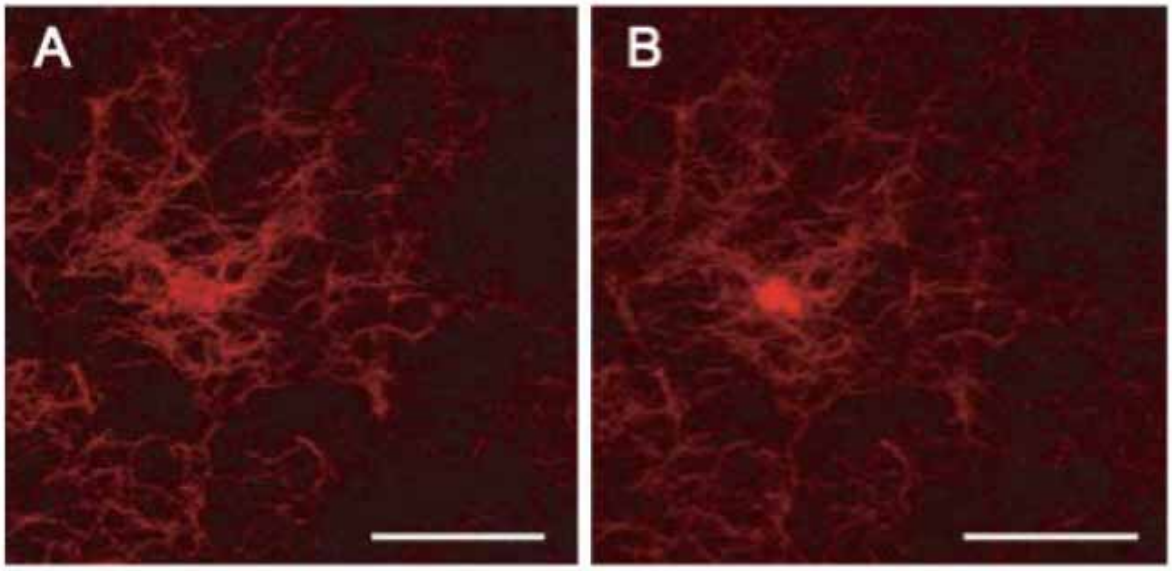

FIG. S4: Confocal images (projections of 30 μm height) of an actin/*α*-actinin network (c_a = 4.25 μM and R = 0.5) at 10 ◦C (A) and 30 ◦C (B). Scale bars denote 50 μm.

Long time equilibration of an actin/α-actinin network

An actin/α-actinin bundle cluster network (ca = 9.5 μM and R = 0.5) is polymerized at 12 °C and the macroscopic plateau modulus G_0 is recorded for several hours. An equilibration of the network resulting in a significant increase of the network stiffness by a factor ≈ 5 is observed (Fig. S5). This is consistent with the increase in the plateau elasticity shown in Fig. 6 of the manuscript.

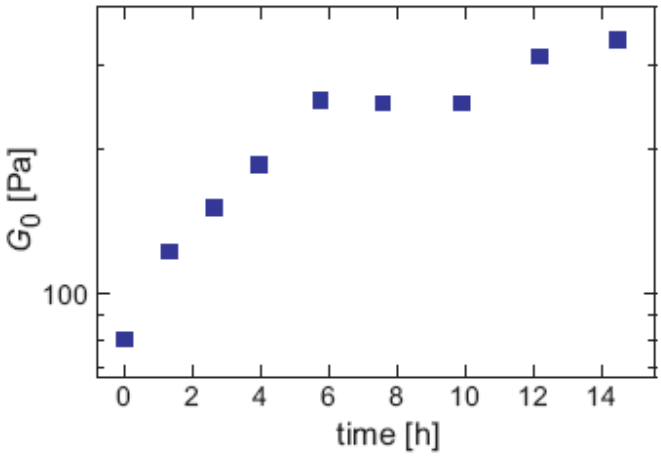

FIG. S5: The plateau modulus G_0 of an α-actinin bundle cluster network (R = 0.5) is shown as a function of the time after full polymerization.

Thermal curing at a higher initial temperature

The hysteresis behavior and thermal curing demonstrated in the manuscript for an *α*-actinin/actin network in the bundle cluster phase is independent from the initial temperature. The same effects as observed for an initial temperature of 12 °C can be reproduced if a higher initial temperature is chosen as depicted in Fig. S6A-D. Please note, that the plateau modulus G_0 does not increase further after ≈ 12 h.

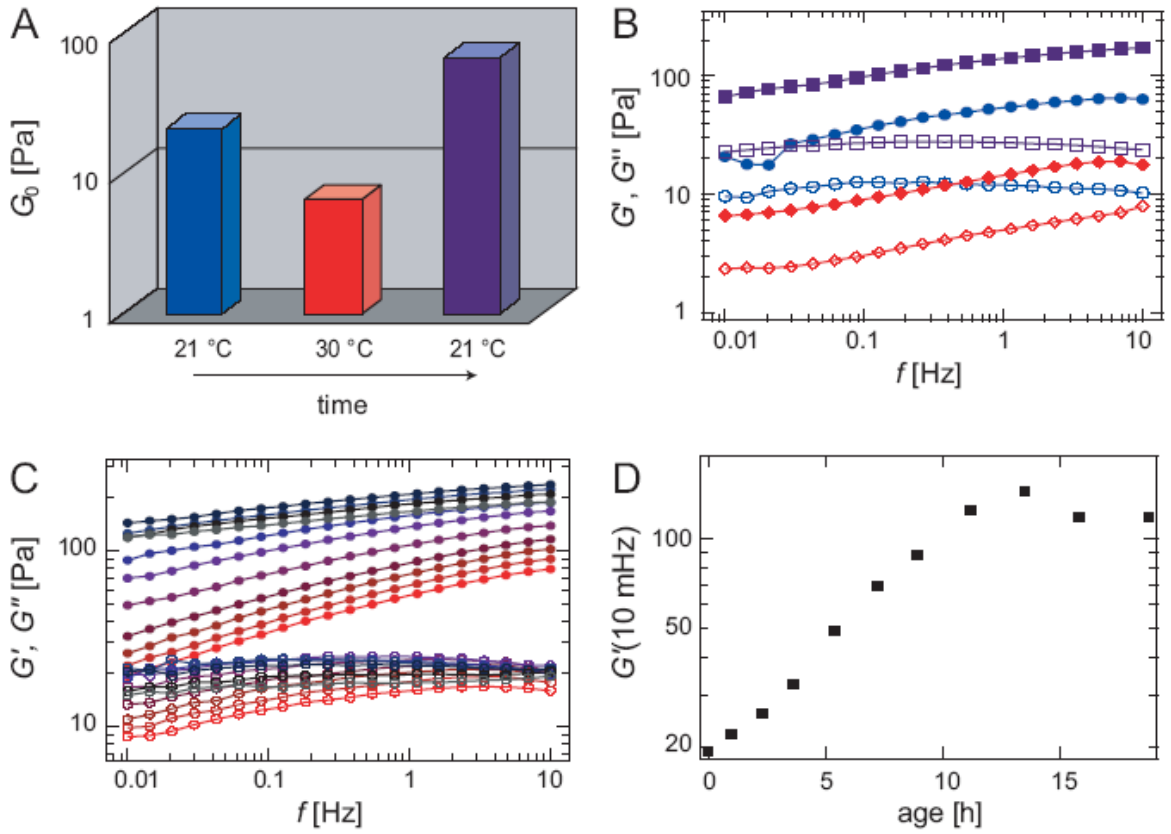

FIG. S6: A) Plateau modulus G_0 at different temperatures: After polymerization at 21 °C (blue) a heating step up to 30 °C (red) followed by re-cooling to 21 °C (purple) was applied. (B) The frequency spectra corresponding to the plateau moduli shown in (A) are compared directly after polymerization at 21 °C (blue circles), after heating to 30 ◦C (red diamonds) and after re-cooling to 21 °C (purple squares). Closed symbols denote *G'(f)*, open symbols denote *G''(f)*. (C) Retarded temporal equilibration at 21 °C. (D) The plateau modulus G_0 as determined from the spectra in (C) is shown as a function of the sample age.

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Influence of the cross-link density on the network elasticity of homogeneous or heterogeneous bundle networks

Using finite element simulations the influence of the cross-link density on the elasticity of bundle networks is investigated. Homogeneous networks of stiff beams are compared to a heterogeneous cluster network. As depicted in Fig. S7, heterogeneous networks are more sensitive to a change in the cross-link density. This can be attributed to the effective loss of 'mechanically active' material if the bundle cluster is increasingly decoupled from the surrounding network by a decrease in the network interconnectivity.

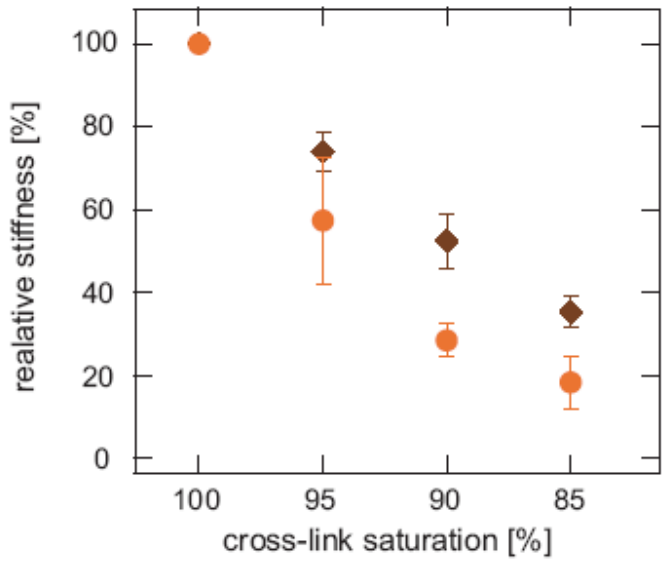

FIG. S7: Finite element simulations of cross-linked bundle networks with decreasing cross-link density: Circles denote networks with a high degree of heterogeneity (30 % of the material is redistributed into one central cluster), diamonds denote homogeneous networks.
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