Supporting Information - Keratins determine network stress responsiveness in reconstituted actin-keratin filament systems

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SUPPLEMENTARY TEXT

The glassy wormlike chain model. The GWLC model used for this study has been comprehensively described previously.[1-3] The GWLC is an extension of the wormlike chain (WLC) for semiflexible polymer networks. The interactions of a test chain with its environment is taken into account by stretching the mode relaxation spectrum of the WLC exponentially. The mode relaxation times of all eigenmodes of (half-) wavelength $\lambda_n = L/n$ and mode number n for a WLC with persistence length l_p and the transverse drag coefficient $\zeta_\perp$ are given by

$$\tau_n^{WLC} = \zeta_\perp/(l_p k_B T \pi^4/\lambda_n^4 + f\pi^2/\lambda_n^2) \quad (1)$$

The relaxation times of the GWLC are modified according to

$$\tau_n^{GWLC} = \begin{cases} \tau_n^{WLC} & if\ \lambda_n \le \Lambda \\ \tau_n^{WLC} e^{\varepsilon N_n} & if\ \lambda_n > \Lambda, \end{cases} \quad (2)$$

where $N_n = \lambda_n/\Lambda - 1$ is the number of interactions per length λ_n, with Λ the average distance between two interaction points, L the contour length of the test filament, the stretching parameter ε controlling how strong the modes are slowed down by interactions with its environment, and f that describes a homogeneous backbone tension accounting for existing pre-stress. The complex linear shear modulus in the high-frequency regime is given by

$$G^*(\omega) = \Lambda/(5\xi^2\chi(\omega)) \quad (3)$$

where ξ is the mesh size of the network. The micro-rheological, linear response function $\chi(\omega)$ to a point force at the ends of the GWLC is calculated as

$$\chi(\omega) = \frac{L^4}{\pi^4 l_p^2 k_B T} \sum_{n=1}^{\infty} \frac{1}{(n^4 + n^2 f/f_E)(1 + i\omega\tau_n^{GWLC}/2)} \quad (4)$$

with the Euler buckling force $f_E = l_p k_B T \pi^2/L^2$. For the linear regime, f is set to zero.

In the nonlinear regime, the differential shear modulus $K = d\sigma/d\gamma$ is approximated via Eq. (3) at a constant frequency as a function of the backbone tension f:

$$K(f) = |G^*_\omega|(f) \tag{5}$$

where f is related to the macroscopic stress σ via $f = 5\sigma\xi^2$. The effect of pre-stress on the stretching parameter is introduced via a linear barrier height reduction:

$$\varepsilon \rightarrow \varepsilon - f\delta/k_BT \tag{6}$$

where δ should be interpreted as an effective width of a free energy well. The mean values of ξ, Λ, and ε obtained from fitting the linear regime for each polymer type were used to replicate the measured curves. δ was used as the only free parameter to effectively shift the peak of K both in terms of σ and the maximum value K_{max}.

SUPPLEMENTARY FIGURES

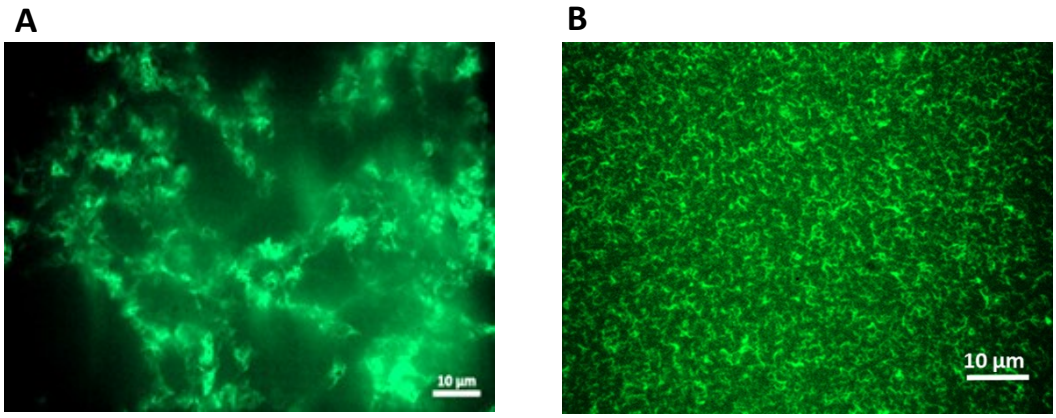

Fig. S1. Confocal micrographs of in situ formed keratin networks assembled in different salt conditions. (A) In F-buffer, a bundled network is formed even at low protein concentration (0.1 mg/ml). (B) In standard low-salt buffer (10 mM Tris pH 7), an isotropic network is formed.

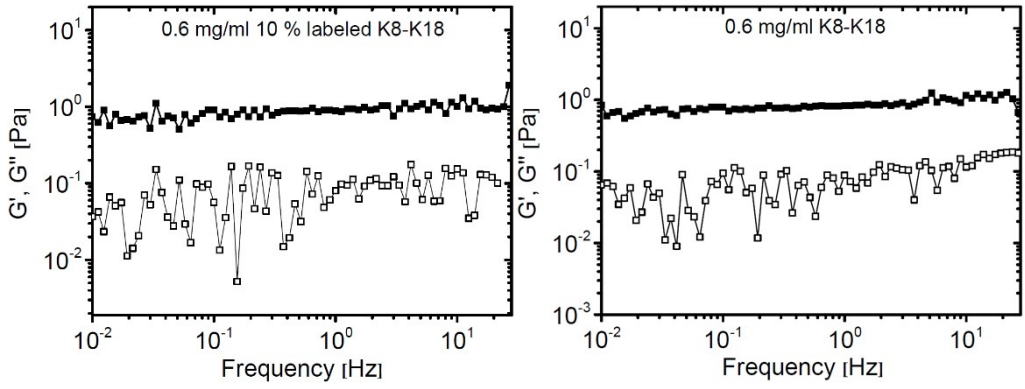

Fig. S2. Linear viscoelastic properties of 10% labeled keratin (0.6 mg/ml) assembled in F-buffer. The labeled fraction did not affect the filaments and network properties. Data points are the mean of three independent measurements.

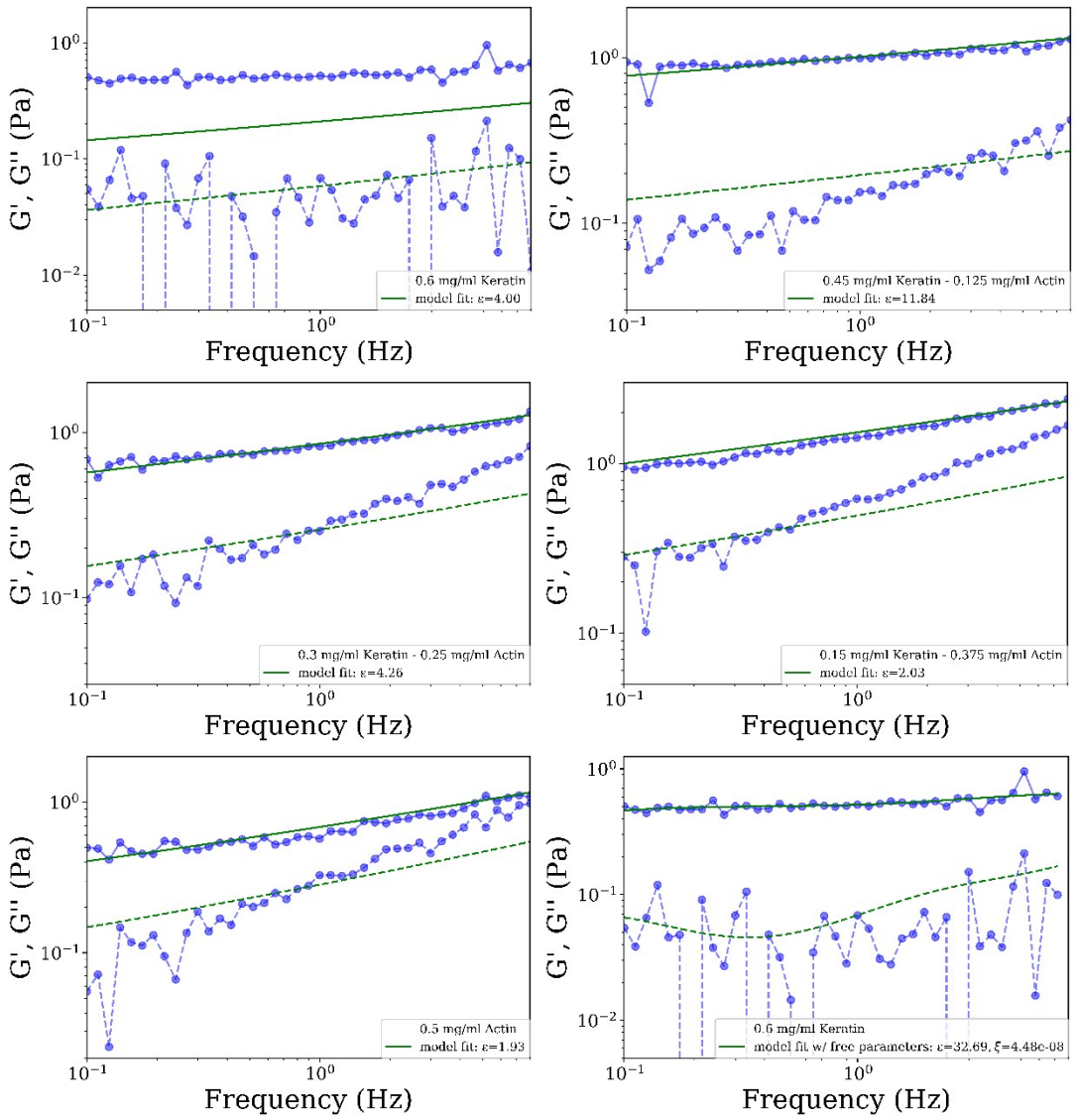

Fig. S3. Means of elastic moduli (solid blue) and loss moduli (dashed blue). GWLC model fits for keratin-actin composite networks in compositions of 0.6 mg/ml keratin (top left), 0.25 mg/ml actin (middle left), 0.15mg/ml keratin-0.375 mg/ml actin (middle right), 0.5 mg/ml actin (lower left). Model fit parameters for the composites as well as the pure networks are shown in figure 3A and discussed in the main article. The figure on the lower right illustrates the model fit for pure keratin without fixing the parameters for mesh size ξ and interaction length Λ.

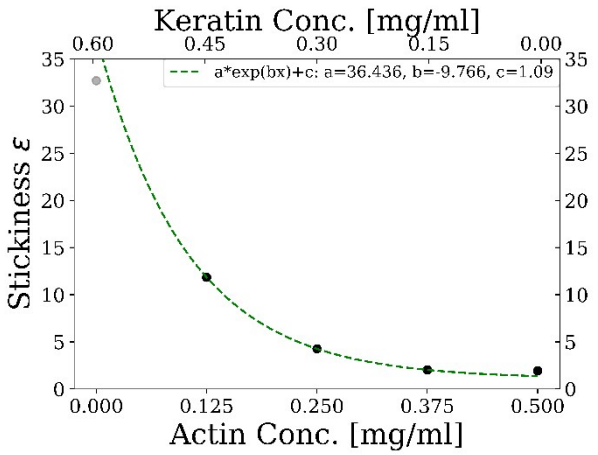

Fig. S4. The stickiness parameter ε shows an exponential decay with increasing actin content in composite keratin/actin networks. Applying a fitting function according to $f(x) = a \times e^{bx} + c$ we obtain $a = 36.436,\ b = -9.766, c = 1.09$. The grey dot represents the epsilon value we obtained for pure keratin with free parameters for mesh size and interaction length (see Fig. S3). It was excluded from the fitting procedure since the experimental conditions were not in accordance with the parameters for the fit. More precisely, the mesh size was treated as a free parameter and orders of magnitude smaller than the experimentally fixed mesh size (see Fig. S3).

SUPPLEMENTARY TABLES

	0.6 mg/ml Keratin	0.45 mg/ml Keratin - 0.125 mg/ml Actin	0.3 mg/ml Keratin - 0.25 mg/ml Actin	0.15 mg/ml Keratin - 0.375 mg/ml Actin	0.5 mg/ml Actin
0.6 mg/ml Keratin	-	p=0.114	p=0.002	p=0.000	p=0.003
0.45 mg/ml Keratin - 0.125 mg/ml Actin	p=0.114	-	p=0.004	p=0.000	p=0.005
0.3 mg/ml Keratin - 0.25 mg/ml Actin	p=0.002	p=0.004	-	p=0.012	p=0.034
0.15 mg/ml Keratin - 0.375 mg/ml Actin	p=0.000	p=0.000	p=0.012	-	p=0.255
0.5 mg/ml Actin	p=0.003	p=0.005	p=0.034	p=0.255	-

Table S1. Significance levels for loss factors. P-values obtained from a Mann–Whitney *U* test.

SUPPORTING REFERENCES

1. T. Golde, C. Huster, M. Glaser, T. Handler, H. Herrmann, J. A. Kas and J. Schnauss, *Soft Matter*, 2018, **14**, 7970-7978.
2. K. Kroy and J. Glaser, *New. J. Phys.*, 2007, **9**, 416-416.
3. T. Golde, M. Glaser, C. Tutmarc, I. Elbalasy, C. Huster, G. Busteros, D. M. Smith, H. Herrmann, J. A. Käs and J. Schnauß, *Soft Matter*, 2019, **15**, 4865-4872.