Supporting Information – Structural Features and Nonlinear Rheology of Self-Assembled Networks of Crosslinked Semiflexible Polymers

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S1. CALCULATION OF THE CONNECTIVITY AND AVERAGE CONTOUR LENGTH

To estimate the connectivity of a given assembled structure, we consider a reduced network defined by (1) merging each pair of crosslinked "linker" nodes into a single vertex and then (2) merging each filament section separating two vertices into a single edge. This is sketched in Fig. S1.

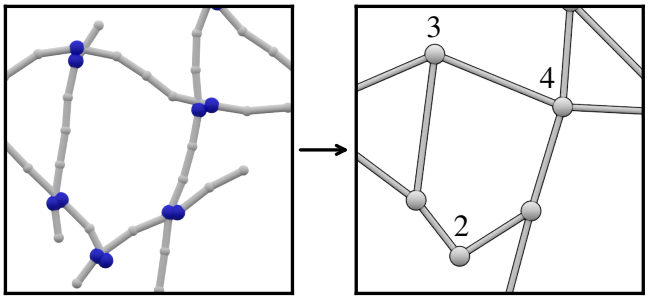

FIG. S1. To calculate a network's average connectivity z, we consider a reduced network structure that treats each crosslink as a node and each inter-crosslink (non-dangling) filament section as an edge. On the right, a few example nodes in the reduced network are labeled according to their connectivity.

S2. PARAMETERS

In the interest of drawing comparison to experimental results in Ref. 1, we have chosen parameter values roughly appropriate for F-actin in laboratory conditions. Specific values are listed in Table S1.

quantity	symbol	value
number of filaments	N_f	500
filament length	ℓ_f	9 μm
filament length per volume	ρ	2.6 μm^{-2}
number of nodes per filament	n	10
filament persistence length	ℓ_p	17 μm
crosslink rest length	$\ell_{0,c\ell}$	0.2 μm
filament stretching rigidity	μ	588 pN
thermal energy scale	k_BT	4.11×10^{-3} pN · μm
solvent viscosity	η_s	0.001 pN · μm^{-2} · s
timestep	Δt	9.42×10^{-7} s
total time, assembly	τ_a	60 s
total time, rheology	τ_{tot}	30 s

TABLE S1. Independent parameters, in real units.

quantity	symbol	value
filament bond rest length	$\ell_0 = \ell_f/(n-1)$	1 μm
simulation box edge length	$L = (N_f \ell_f/\rho)^{1/3}$	12 μm
filament bending rigidity	$\kappa = k_B T \ell_p$	6.99×10^{-2} pN · μm^2

TABLE S2. Parameter-dependent quantities.

Assuming a molecular mass of 42 kDa for actin[2], the mass per length is $\sim 16{\times}10^3$ kDa/μm, which translates to 0.63 μmol/μm. Therefore, our chosen length density of $\rho = 2.6$ μm^{-2} corresponds to an actin concentration of $c_A = 1.63$ μM.

Quantities used in simulations are nondimensionalized by characteristic length, force, and drag coefficient values: $\ell^* = 1$ μm, $f^* = 1$ pN, and $\zeta^* = 6\pi\eta_s(\ell_0/2) = 9.42 \times 10^{-3}$ pN · μm · s (for example, the simulation timestep is $\Delta t_{\text{sim}} = \Delta t/(\zeta^* \ell^*/f^*) = 10^{-4}$. Note that one can define a dimensionless bending rigidity $\tilde{\kappa} = \kappa/(\mu \ell_0^2)$, as in past work.[3] With our parameters, $\tilde{\kappa} = 1.18 \times 10^{-4}$.

S3. FITS OF K TO THE EXPECTED SCALING FORM

We assume that the differential shear modulus $K = \partial\sigma/\partial\gamma$ can be written as

$$K = a\mathcal{K}(\tilde{\kappa}, \gamma) \tag{S1}$$

in which a is some prefactor with units of stress, $\tilde{\kappa}$ is a dimensionless bending rigidity, and $\mathcal{K}$ is a dimensionless function of strain that scales with the distance to a critical strain γ_c as[3]

$$\mathcal{K} \propto |\gamma - \gamma_c|^f \mathcal{G}_{\pm}\left(\frac{\tilde{\kappa}}{|\gamma - \gamma_c|^{\phi}}\right). \tag{S2}$$

This scaling is reproduced by the constitutive equation

$$\frac{\tilde{\kappa}}{|\gamma - \gamma_c|^{\phi}} \sim \frac{\mathcal{K}}{|\gamma - \gamma_c|^f}\left(\pm 1 + \frac{\mathcal{K}^{1/f}}{|\gamma - \gamma_c|}\right)^{\phi - f} \tag{S3}$$

in which the plus and minus correspond to the regions below and above the critical strain, respectively. We compute $\mathcal{K}(\gamma)$ by numerical inversion of $\tilde{\kappa}(\mathcal{K})$:

$$\tilde{\kappa} = b\mathcal{K}\left(\pm|\gamma - \gamma_c| + \mathcal{K}^{1/f}\right)^{\phi - f}, \tag{S4}$$

setting $b = 1$.

For each set of measured $K(\gamma)$, we determine the best-fit parameters $\{\tilde{\kappa}, \gamma_c, f, \phi, a\}$ by performing a fit of $\log_{10}(a\mathcal{K}(\gamma))$ to $\log_{10}(K(\gamma))$ using the non-linear least squares fitting function `scipy.optimize.curve_fit` from the open source Python library SciPy[4]. Best-fit parameters for the data in Fig. 7a in the main text are shown in Fig. S2. We note that one can alternatively compute the critical strain γ_c as the value of γ corresponding to the inflection point of $\log_{10} K$ vs. $\log_{10} \gamma$,

$$\gamma_c = \text{argmax}\frac{d\log_{10} K}{d\log_{10}\gamma} \tag{S5}$$

as has been done in related work[3]. In Fig. S2e, we show that the values of γ_c determined using the fitting procedure described here agree well with values computed using Eq. S5.

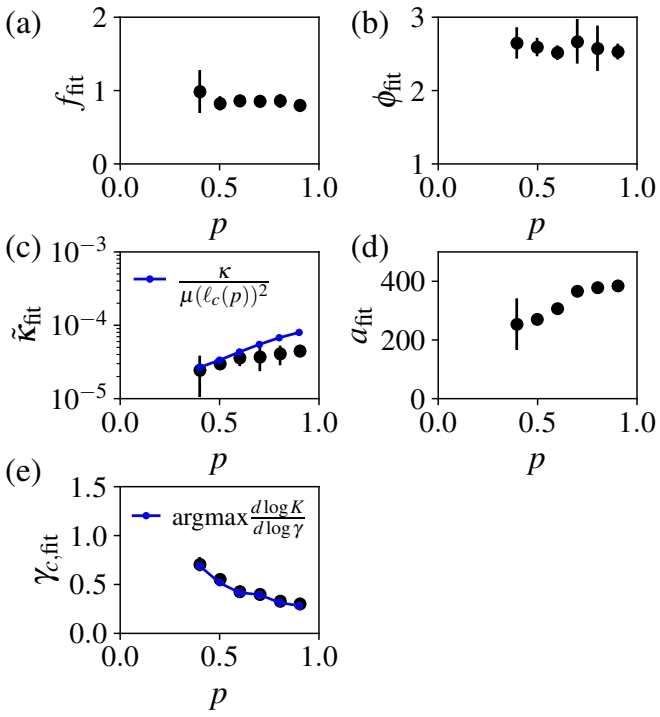

FIG. S2. Best-fit parameters for systems with varying crosslinker coverage fraction p, corresponding to the solid curves in Fig. 7a in the main text.

S4. STOPPING CRITERION FOR NETWORK ASSEMBLY

The rate at which crosslinks are formed slows as the assembly process progresses. To determine an appropriate stopping time for the assembly process, we track the total number of crosslinks formed per volume, $\rho_{c\ell}$, as a function of elapsed assembly time t, as shown in Fig. S3a. We deem network structures "stabilized" if, at time t, the total number of crosslinks formed per volume has increased by less than 1% over the most recent $\alpha = 10^7$ timesteps. Equivalently, we require that $(\rho_{c\ell}(t)-\rho_{c\ell}(t-\alpha\Delta t))/\rho_{c\ell}(t-\alpha\Delta t) \leq 10^{-2}$. In Fig. S3b, we plot the fractional increase, $(\rho_{c\ell}(t) - \rho_{c\ell}(t - \alpha\Delta t))/\rho_{c\ell}(t - \alpha\Delta t)$, as a function of elapsed time. While it is clear that our network stabilization criterion is satisfied by $t/\Delta t \approx 4 \times 10^7$ for the chosen range of parameters, we conservatively select a total assembly time $\tau_{\rm a} = 6 \times 10^7 \Delta t$ for all systems.

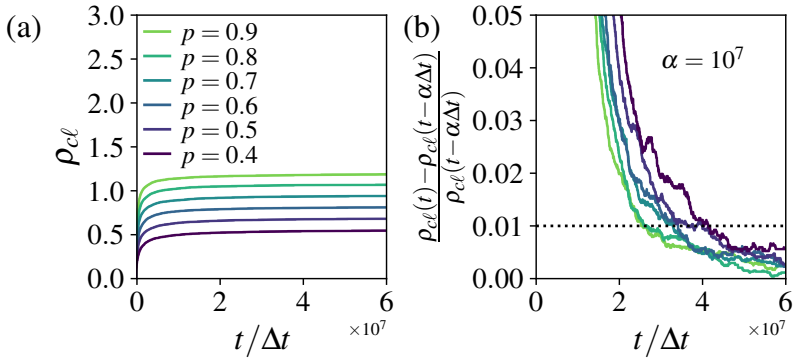

FIG. S3. (a) Total number of crosslinks formed per volume, $\rho_{c\ell}$, as a function of elapsed assembly time. (b) Fractional increase in the total number of crosslinks formed per volume between times $t - \alpha\Delta t$ and t, with $\alpha = 10^7$. The dotted line indicates our network stabilization criterion, $(\rho_{c\ell}(t) - \rho_{c\ell}(t - \alpha\Delta t))/\rho_{c\ell}(t - \alpha\Delta t) \leq 10^{-2}$.

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