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Supporting Material

Actin filament length tunes elasticity of flexibly cross-linked actin networks

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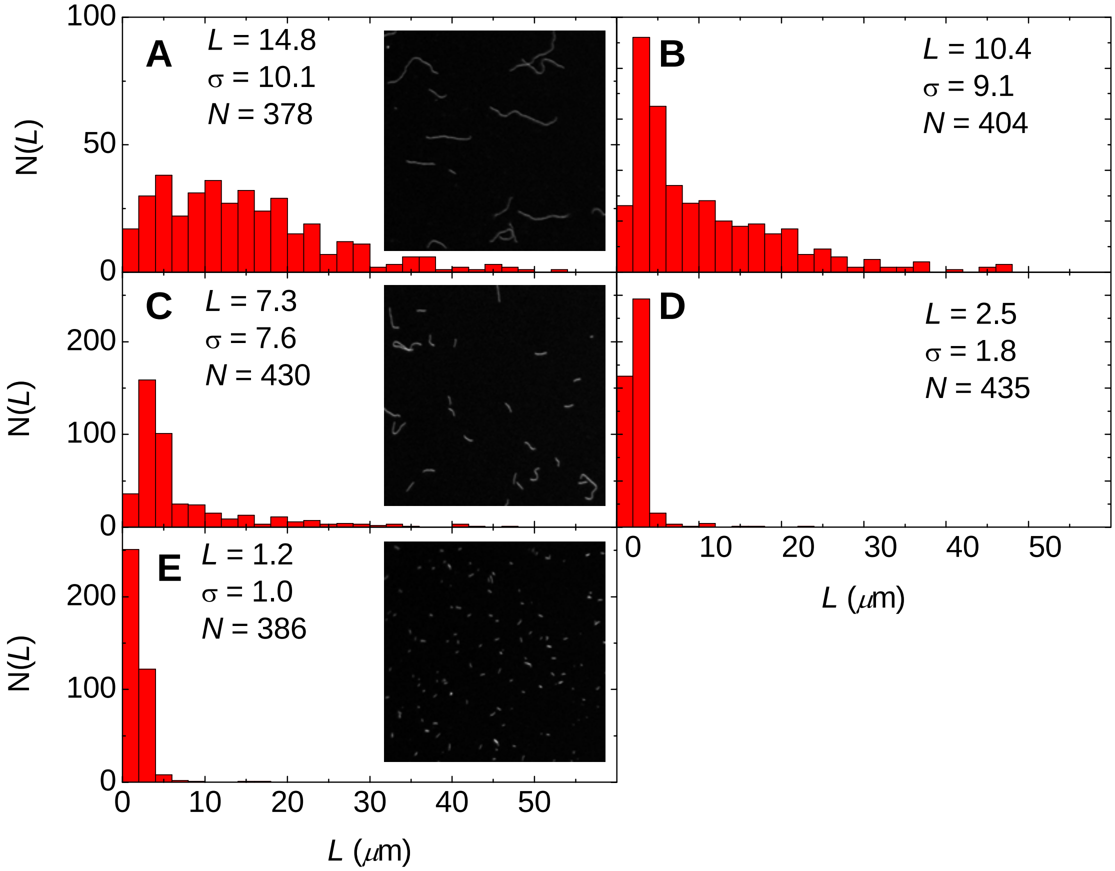
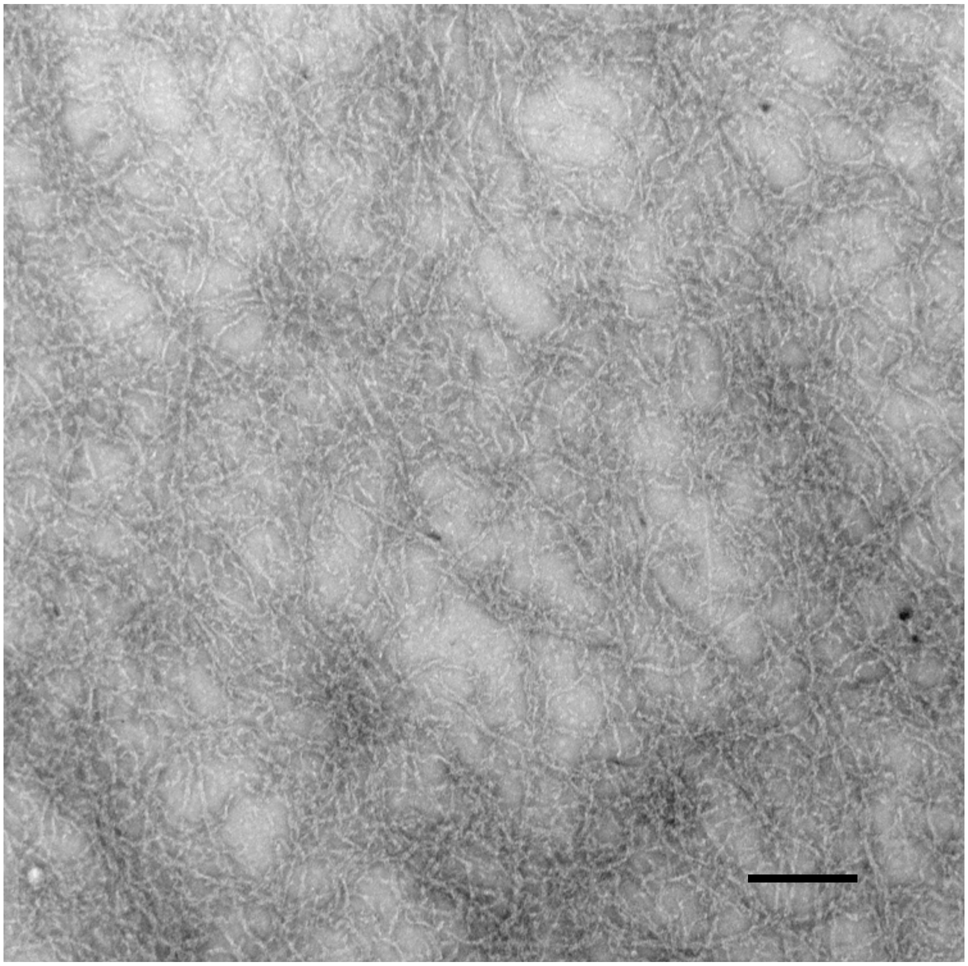
S1. Actin filament length distribution:

[gelsolin]:[actin] = R_G = 0 (*A*), 1:3700 (*B*), 1:1850 (*C*), 1:740 (*D*), and 1:370 (*E*). Image size is 82 μm.

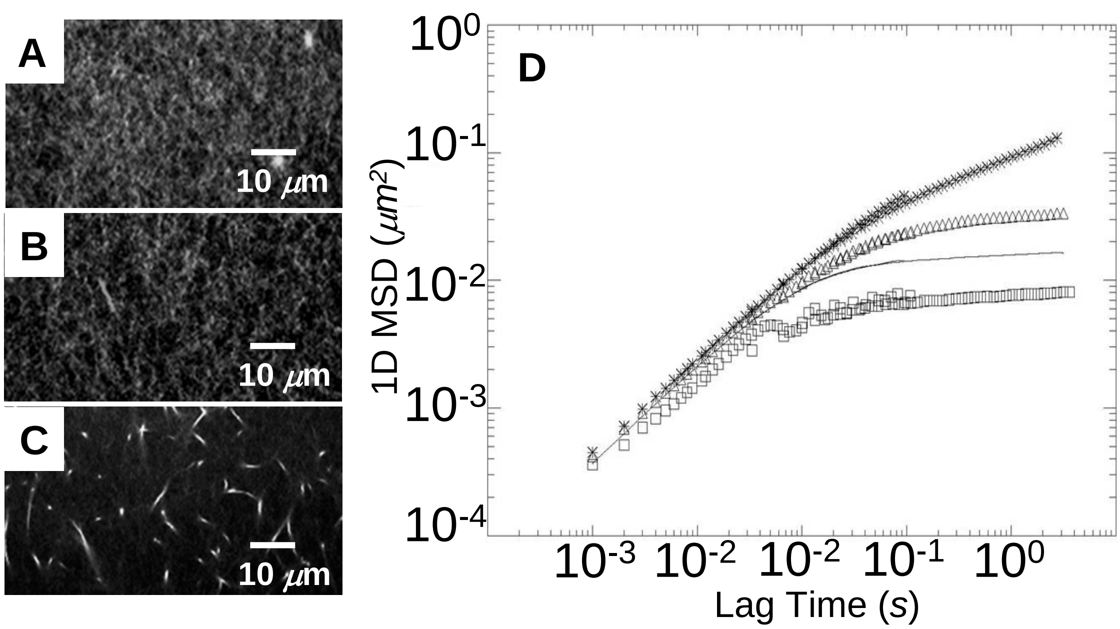


S2. Transmission electron micrograph of F-actin:
[gelsolin]:[actin] = R_G = 0 (L = 15 μm). Without filamin or biotin-NeutrAvidin crosslinks. Scale bar is 300 nm.



S3. We confirm the bundling transition by tracking the thermal motion of 1 micron PEG-coated particles within the networks. For 0.5 mg/ml actin and R_F = 0, where the mesh size is expected to be approximately 400 nm, the particles' ensemble mean-squared-displacement (MSD) reaches a plateau consistent with particles trapped inside an elastic medium with pore size smaller than the particle diameters. For R_F = 0.005, the MSD still reaches a plateau, but the plateau value is decreased compared to R_F = 0, indicating that the mesh size remains smaller than the particle diameter, but now the stiffness of the elastic medium has increased due to crosslinking. In contrast, when we further increase R_F to 0.01, the value of the MSD plateau now increases, suggesting there is an increase in the mesh size. Increasing R_F even further to 0.02, the slope of the MSD increases and never reaches a plateau. At this point, enough actin has partitioned into bundles that the typical mesh size has become larger than the particle diameter.

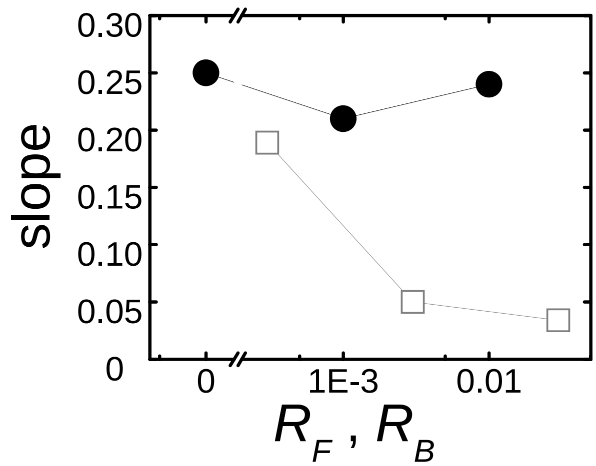
(*A*-*C*) Confocal microscopy of actin-filamin networks.
0.5 mg/ml actin, no gelsolin, various R_F: *A*) R_F = 0.002, *B*) R_F = 0.005, *C*) R_F = 0.01.
D) Multi-particle tracking shows effect of bundling. 0.5 mg/ml actin, no gelsolin, 1 micron PLL-PEG particles; 1-point, 1D MSD, R_F = 0 (*line*), 0.005 (*squares*), 0.01 (*triangles*), 0.02 (*crosses*).



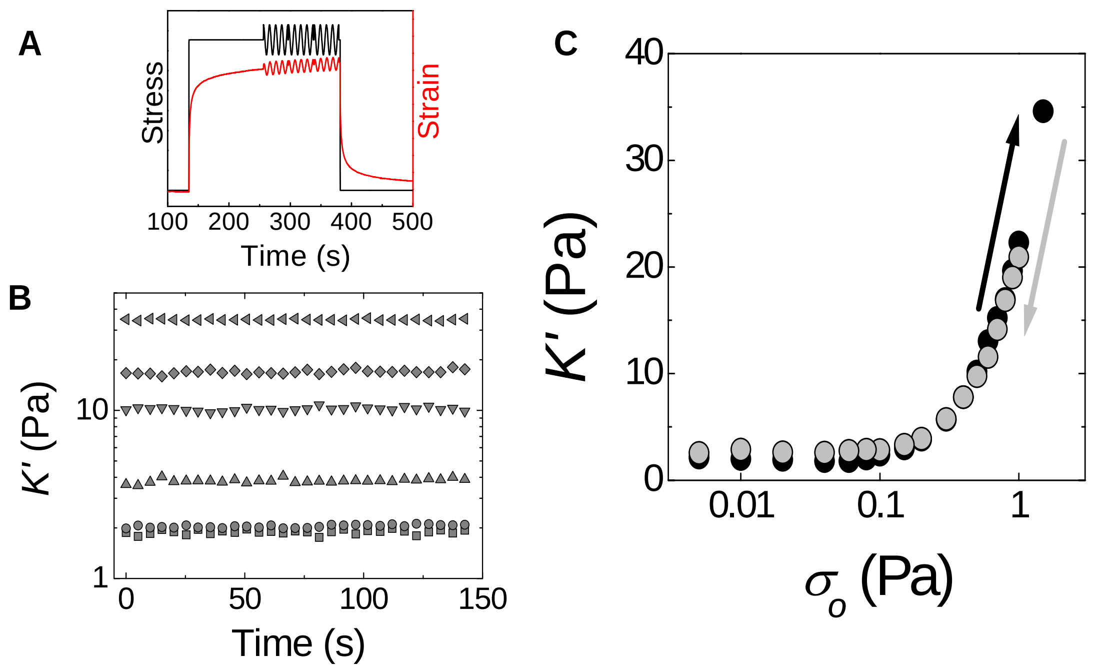
S4. Power-law slope of $G'(\omega)$:

0.5 mg/ml actin, L = 15 microns.

Filamin vs. R_F (*black circles*). Biotin-NeutrAvidin vs. R_B (*gray squares*).



S5. Nonlinear rheology:
Prestress measurements on networks with 1.0 mg/ml actin and R_F = 0.005 filamin.
A) Small oscillatory stress superposed on a steady prestress. Measure oscillatory strain response to determine differential elastic and viscous moduli, K' and K''.
B) No time dependence in K' at various levels of prestress.
C) Minimal hysteresis in K' as prestress is ramped up to just below breakage stress and back down.



S6. Maximum stress and stiffening for crosslinked networks:

A) Maximum stress as a function of R_F for filamin crosslinked networks with L = 15 μm (*black circles*) or L = 5 μm (*black triangles*) and as a function of R_B for rigidly crosslinked networks with L = 15 μm (*gray circles*). Gray line denotes mean maximum stress for rigidly crosslinked networks having $R_B > 0.001$.

B) Magnitude of stiffening as a function of crosslinking ratios for networks with L = 15 μm. Filamin vs. R_F (*black circles*), rigid crosslinks vs. R_B (*gray circles*).

(*A*-*B*) Non-bundled networks (*solid symbols*) and bundled networks (*open symbols*).