

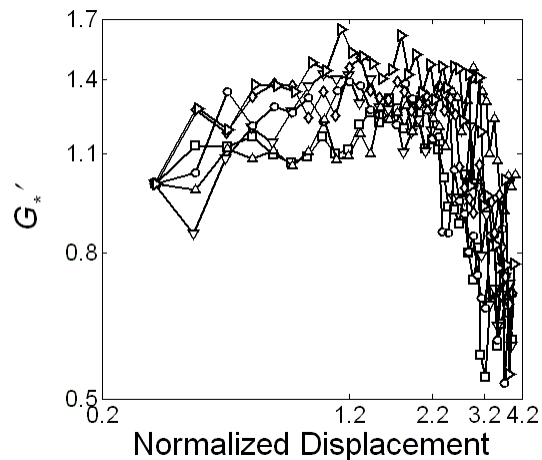
# Supplementary Material for

## Molecular Origin Of Strain-Softening In Cross-Linked F-Actin Networks

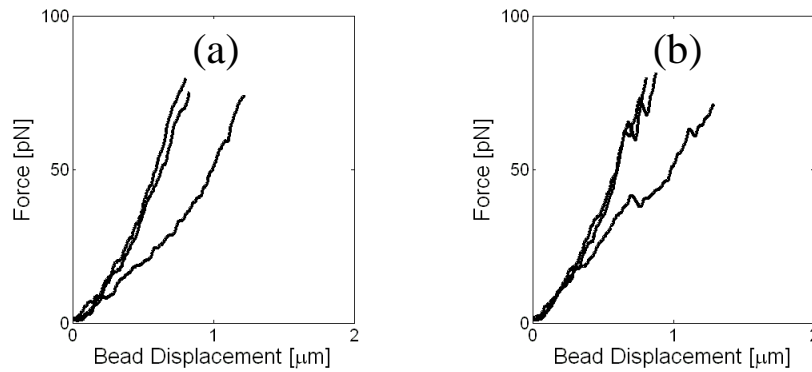
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### Microsphere preparation.

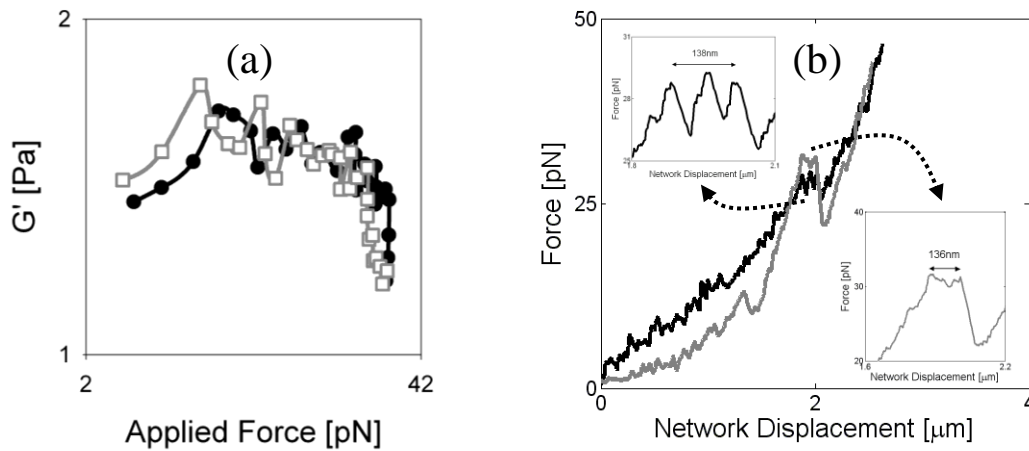
1mg/ml PLL-PEG solution is prepared by dissolving Poly-L-Lysine-graft-PolyEthylene Glycol (PLL-PEG; SurfaceSolutions Inc. Zurich, Switzerland) in G-buffer (5 mM Tris-HCl, 0.2 mM CaCl<sub>2</sub>, 0.5mM DTT, 0.2 mM ATP, pH 8.0). 1 $\mu$ m dia. carboxylated microspheres stock (2.5% solids, Polybead Carboxylate Microspheres; Polysciences, Warrington, PA) is diluted to be 5mg/ml with G-buffer. 100 $\mu$ L of PLL-PEG solution is mixed with the same amount of bead solution for 30min using a rotator. The microsphere solution is centrifuged at 2000g for 15min and resuspended with 100 $\mu$ L G-buffer. After washing for three times, the microsphere pellets are resuspended with 50 $\mu$ L G-buffer. The PEG-coated microspheres are kept at 4°C and used within 10 days.



**Fig. S1** Normalized elastic modulus  $G_*'$  as a function of the normalized bead displacement for the F-actin networks cross-linked with  $\alpha$ -actinin.



**Fig. S2** Representative force-displacement curves without (a) and with (b) a force peak for streptavidin/biotin-F-actin networks. (a) Approximately 69% of the measurements did not show a transition in their force responses. (b) Even in the responses with a force drop, the transitions occur at  $58.7 \pm 9.7$  pN, which is higher than the critical force for filamin networks.



**Fig. S3** (a) Reversible behavior is observed in some measurements of mechanical properties of the filamin/F-actin networks ( $c_A = 30\mu\text{M}$ ,  $R_f = 0.01$ ) for increasing ( $\bullet$ ) and decreasing ( $\square$ ) force. (b) Consecutive undulations (top inset) or a plateau (bottom inset) observed in the network response might be attributed to unfolding of filamin rather than unbinding.