

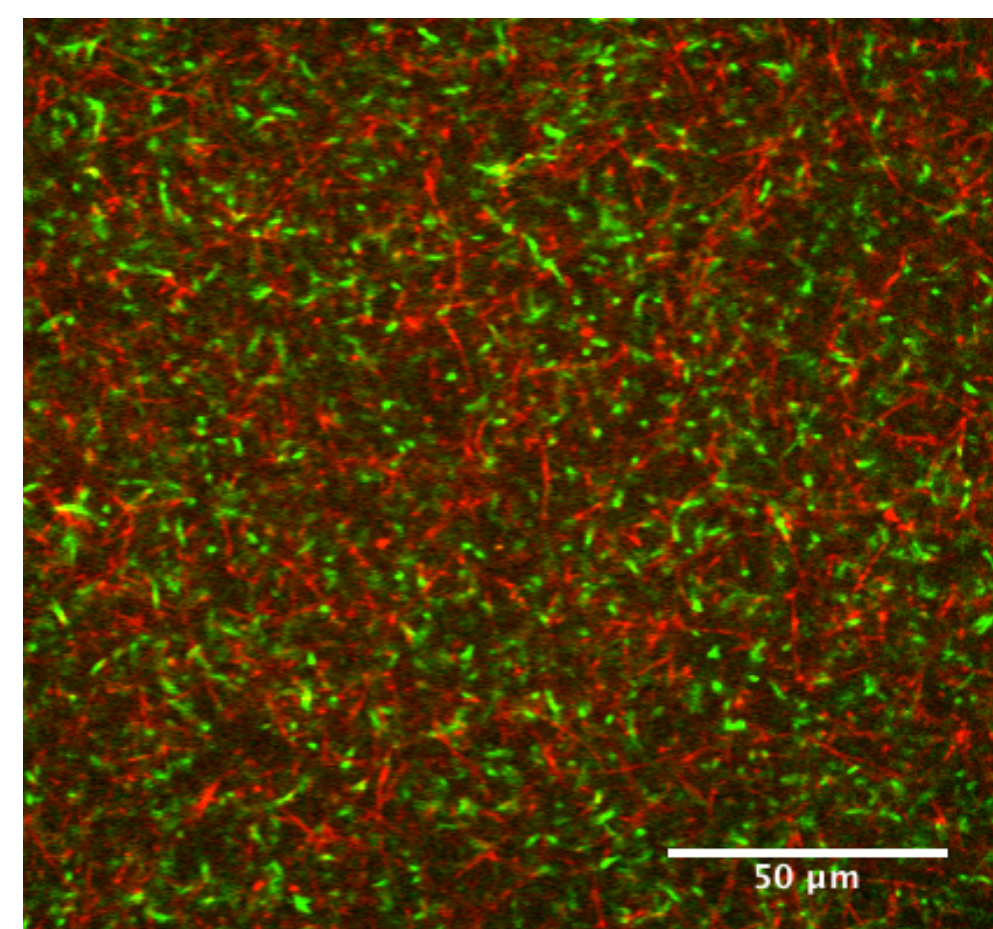
# The effect of varying crosslinking motifs on the microscale mechanics of co-entangled actin and microtubules

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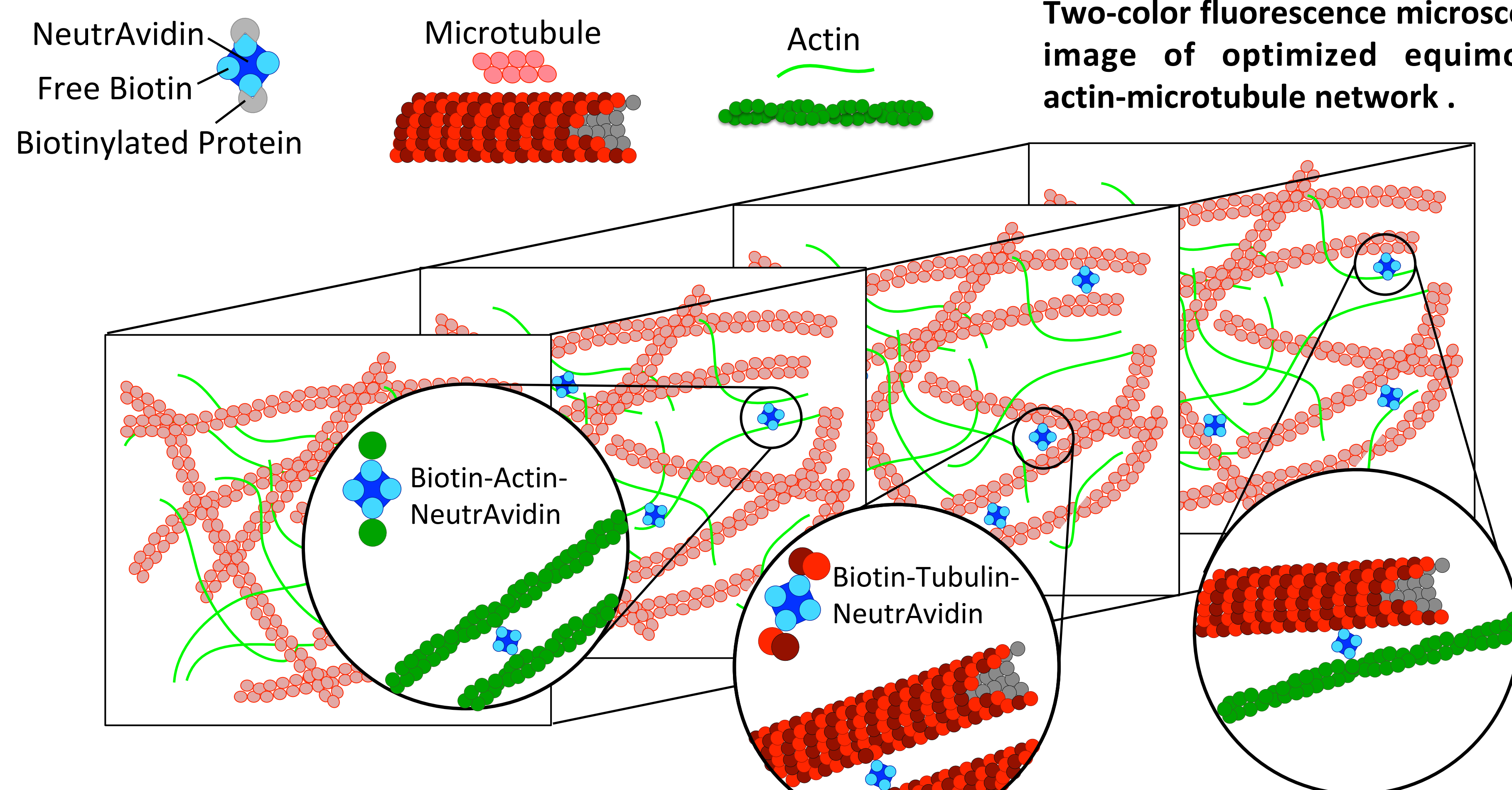
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## Isotropic crosslinked co-entangled actin-microtubule networks

The cytoskeleton is largely comprised of semiflexible actin and rigid microtubules that entangle and interact via smaller crosslinking proteins to form complex networks and structures, giving rise to multifunctional mechanics in cells. Despite actin and microtubules coexisting in cells, the standard in vitro polymerization protocol for the two proteins are incompatible. We designed hybrid buffers and polymerization methods to create stable actin-microtubule networks. Then, we introduced crosslinking using Biotin-NeutrAvidin.

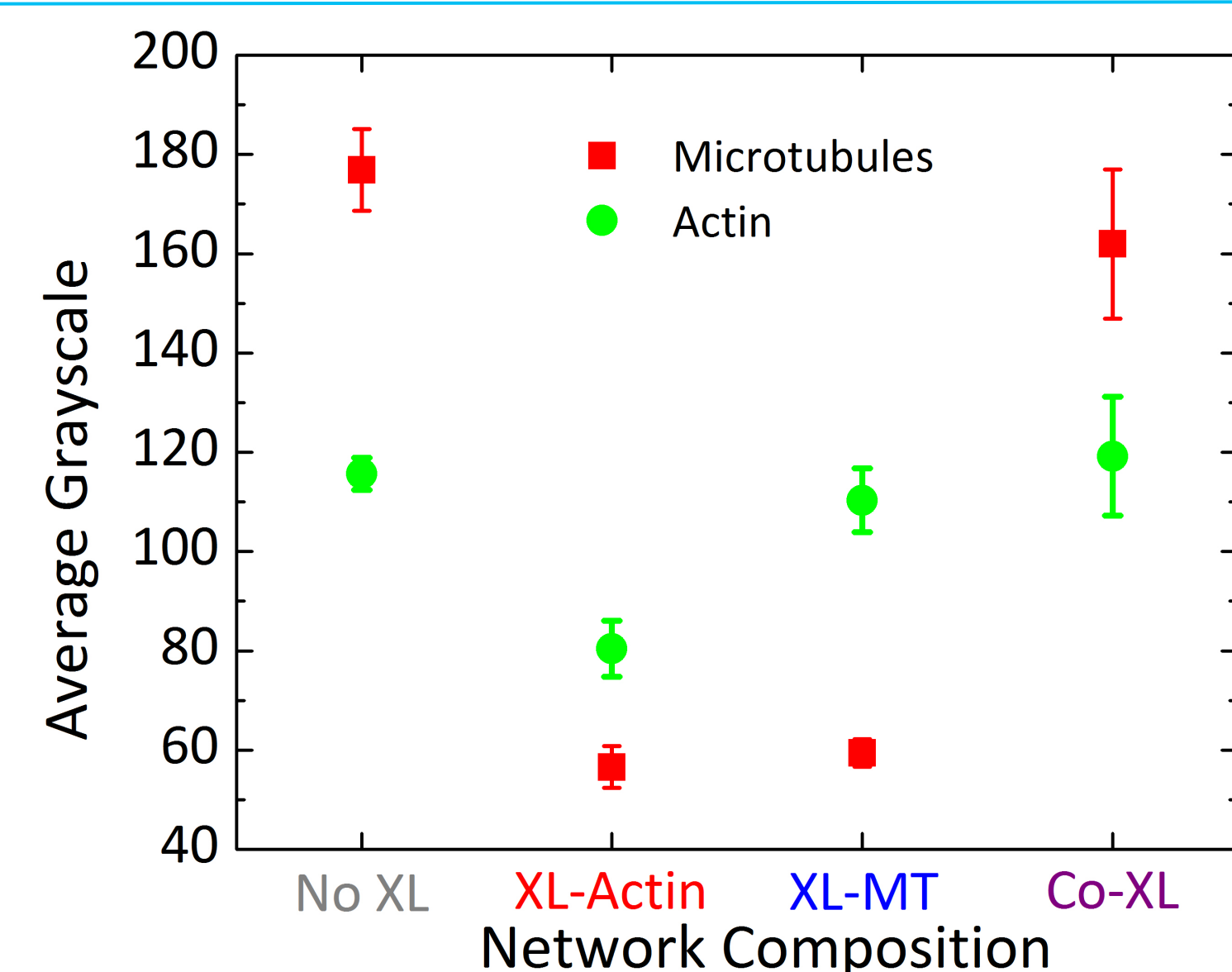


Two-color fluorescence microscopy image of optimized equimolar actin-microtubule network.



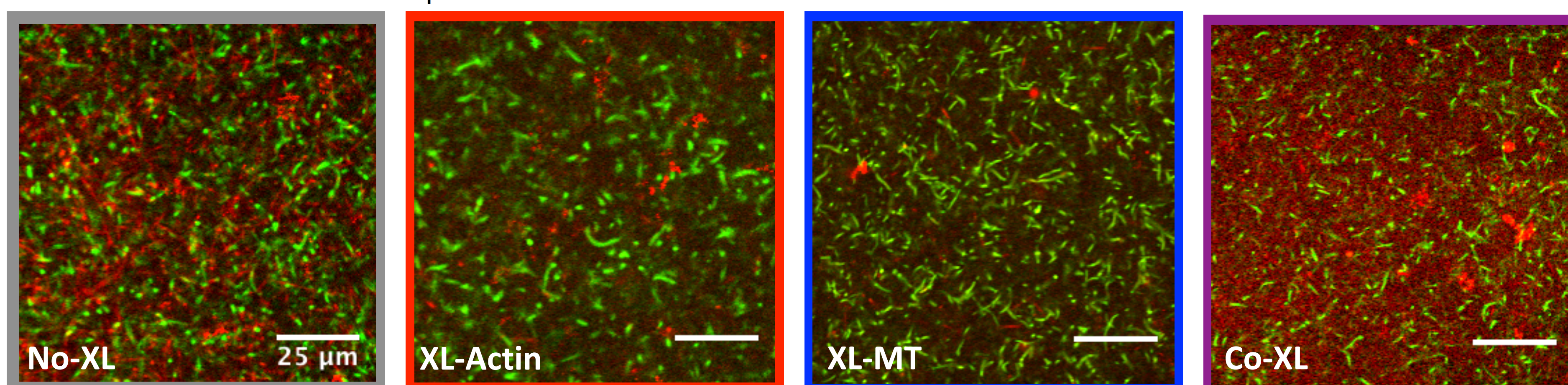
## We create networks with varying crosslinking motifs

All networks are equimolar with a fixed crosslinker density. We create actin-microtubule networks without crosslinkers, one with a fixed number of crosslinker in which all of the actin is crosslinked, a network in which the crosslinker protein crosslinks microtubules and finally a network in which actin and microtubules are co-crosslinked.

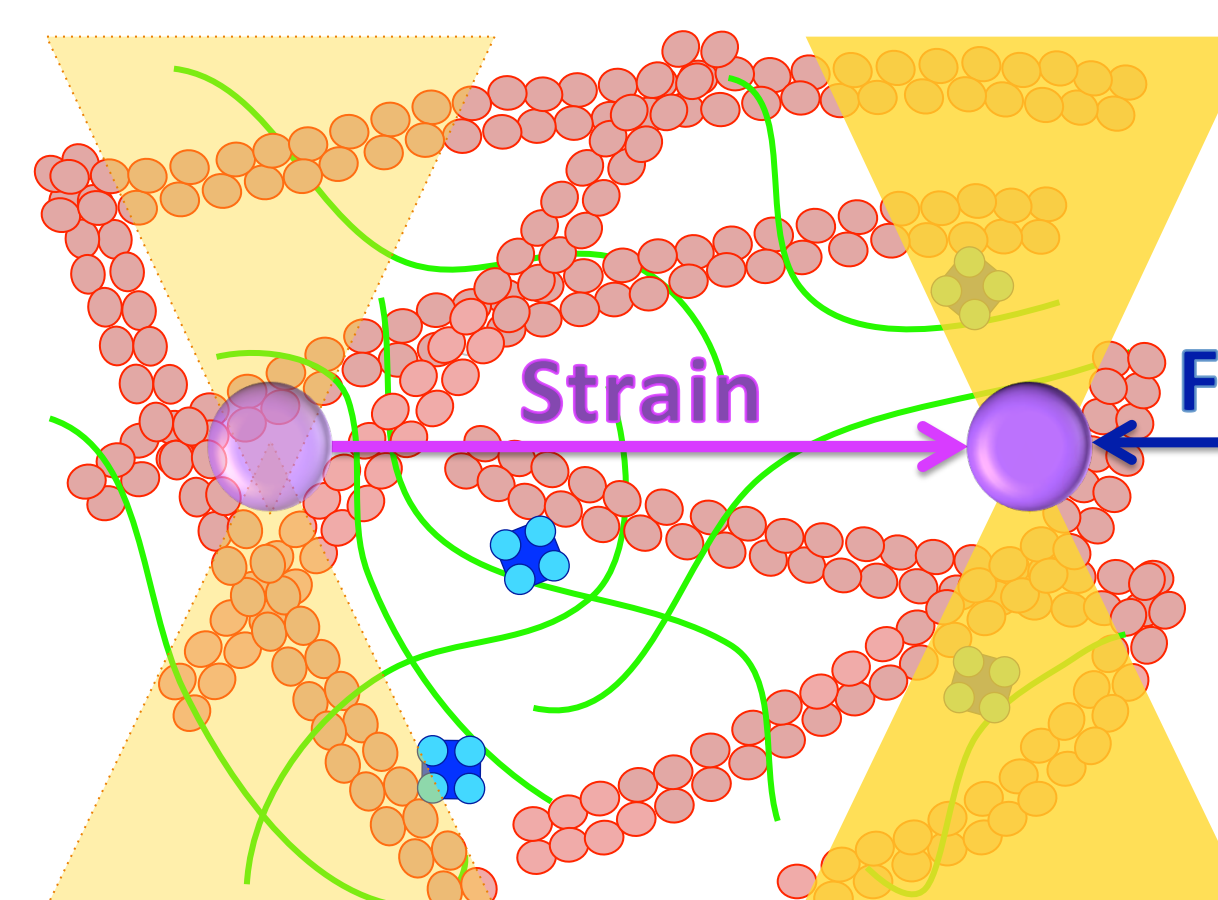


## Confocal imaging reveals the average mobility of actin and microtubules in networks

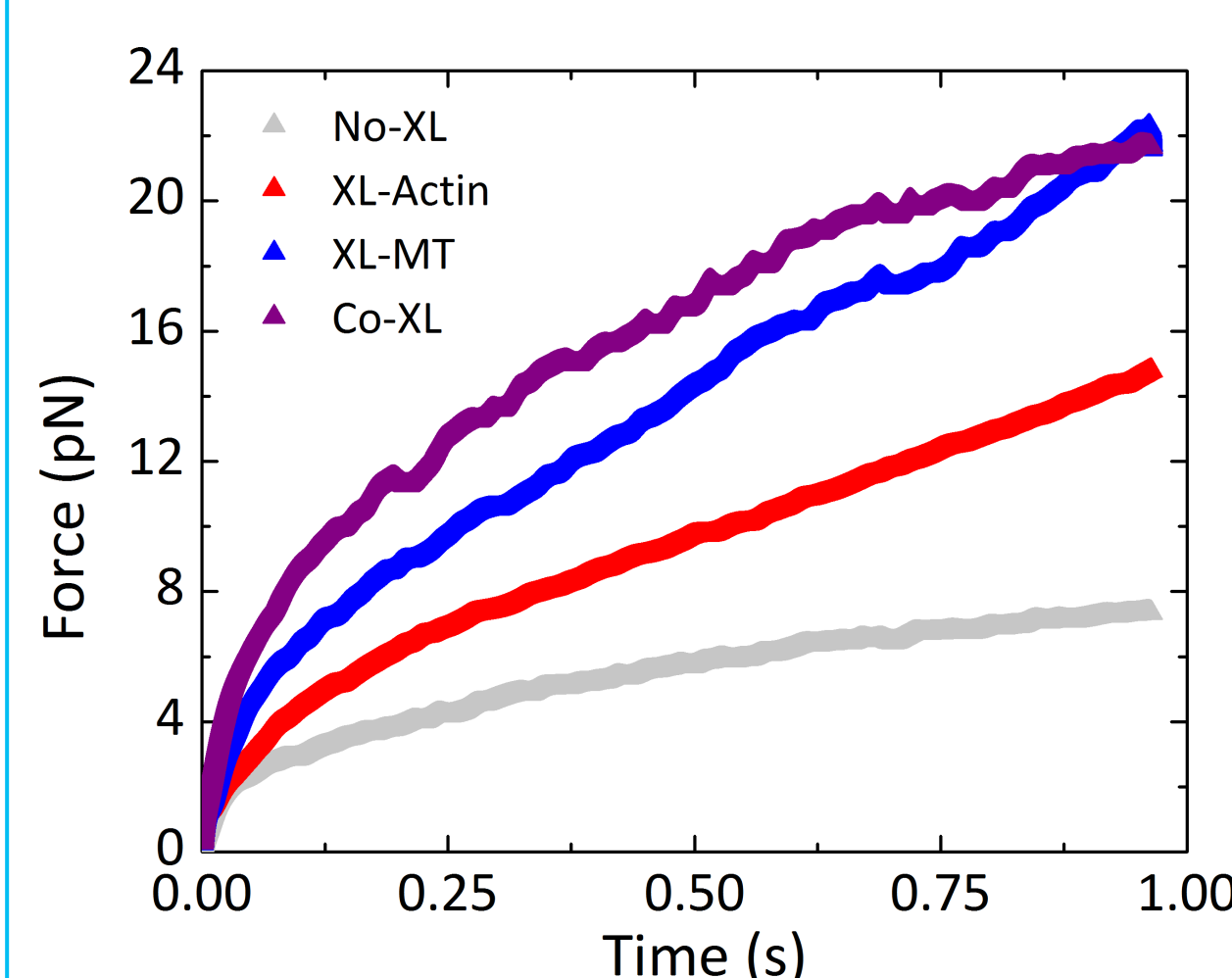
To understand the force response, we look at the network structure and mobility. We collapse time lapse videos into a single image by taking the standard deviation of each pixel. Images that look brighter have a greater standard deviation which means the filaments are fluctuating more and implies more mobility. By looking at the average grey scale value, or pixel intensity, we see the average intensity of actin is largely constant where as in single component networks, the average intensity of the microtubules drops.



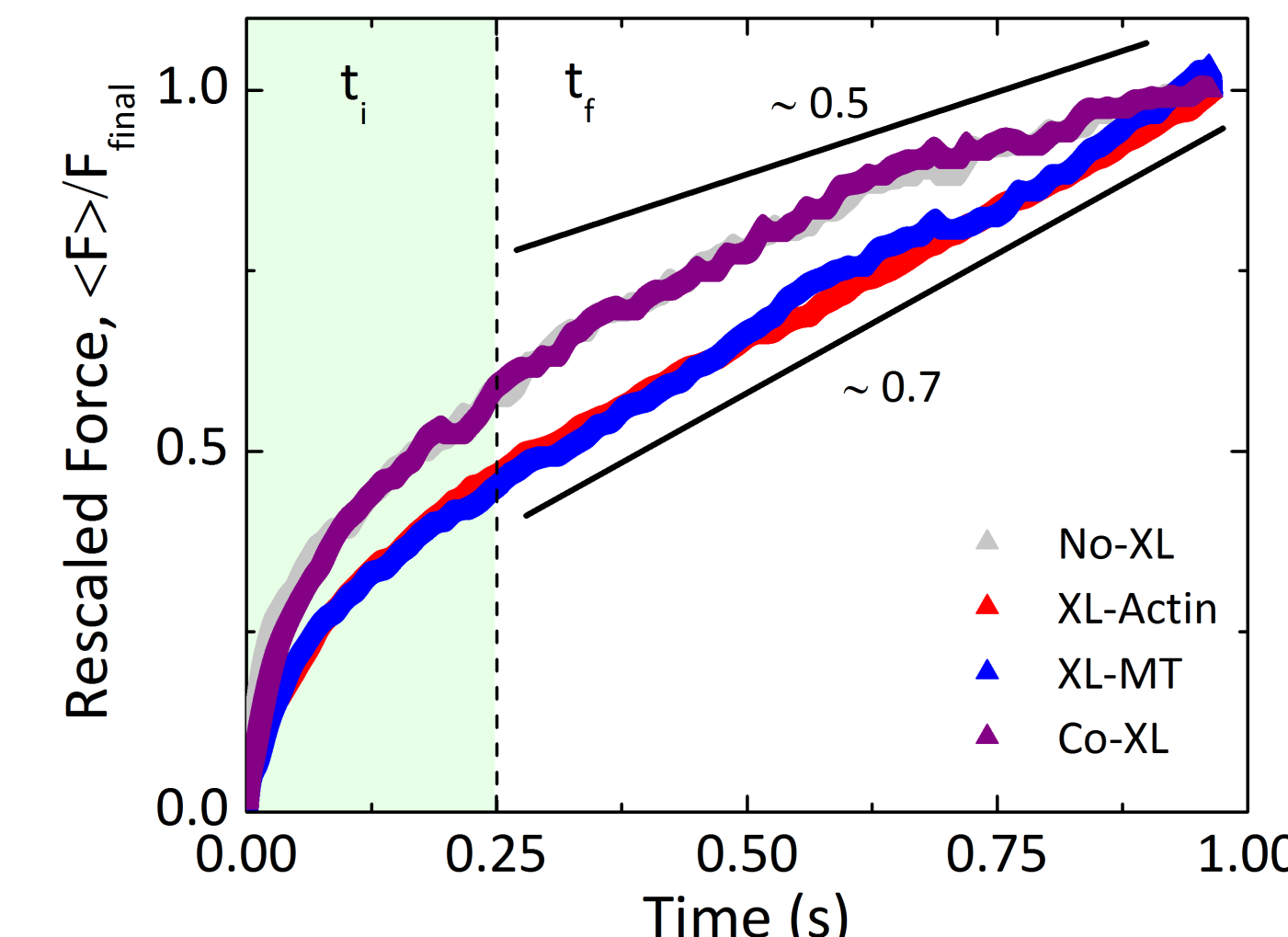
Funding: NSF CAREER Award (DMR-1255-466) and Scialog Collaborative Innovation Award Funded by Research Corporation for Scientific Advancement (grant no. 24192)



**Microscale force measurements of actin-microtubule composites** An optically trapped 4.5 μm microsphere (pink) embedded in a crosslinked actin-microtubule composite is displaced 30 μm at a constant speed of 20 μm/s with optical tweezers. During and following the microsphere movement (strain), the force the composite exerts to resist the strain ( $F$ , blue) is determined by measuring the deflection of the trapping laser, which is proportional to the force.

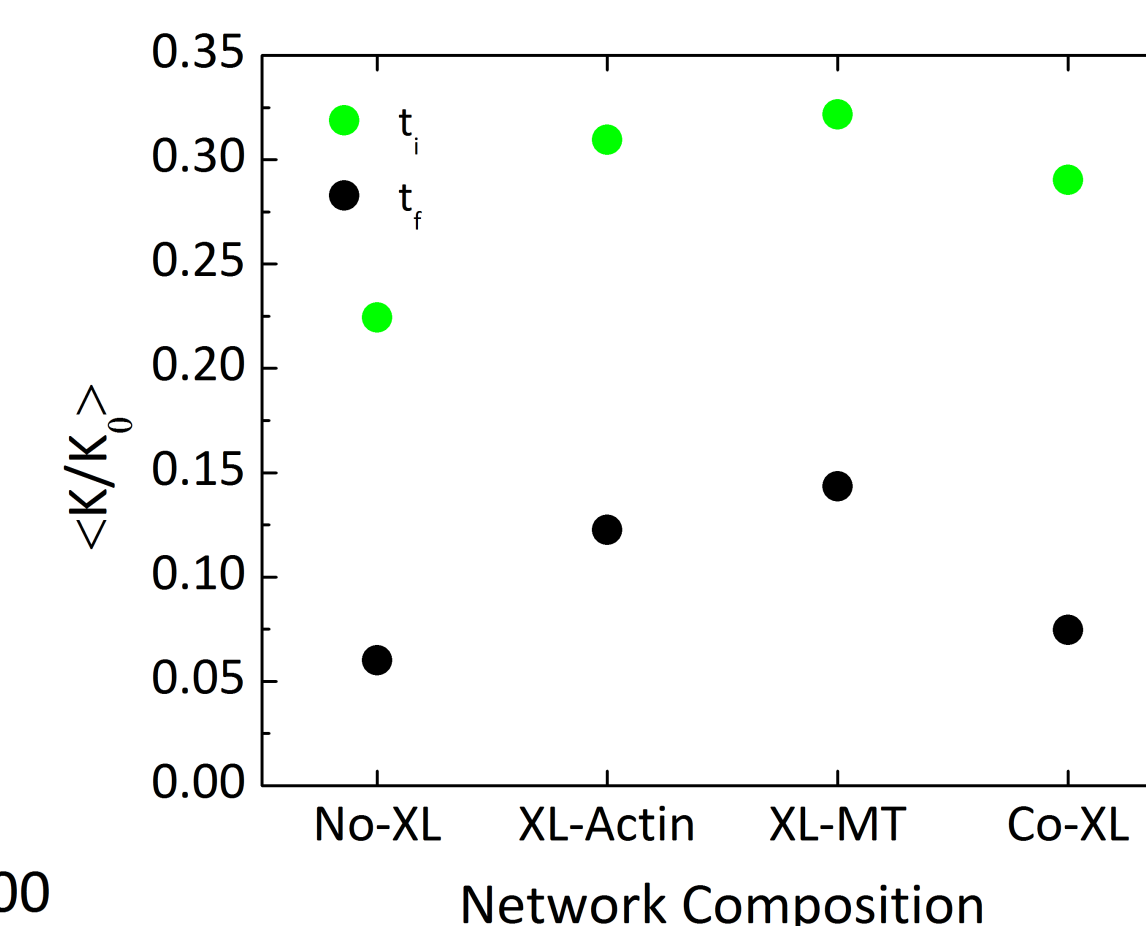


Crosslinking increases resistive force of composites networks

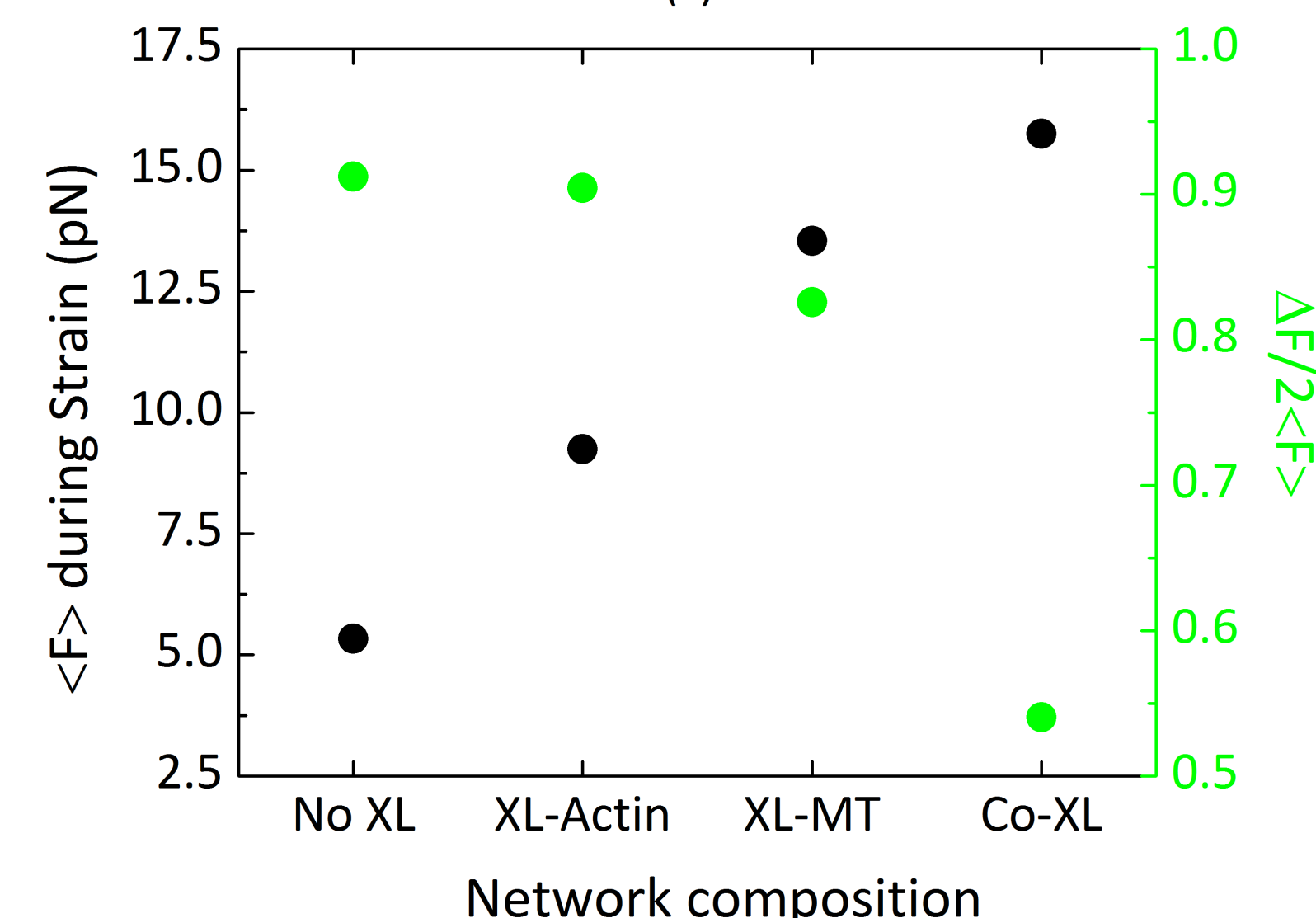
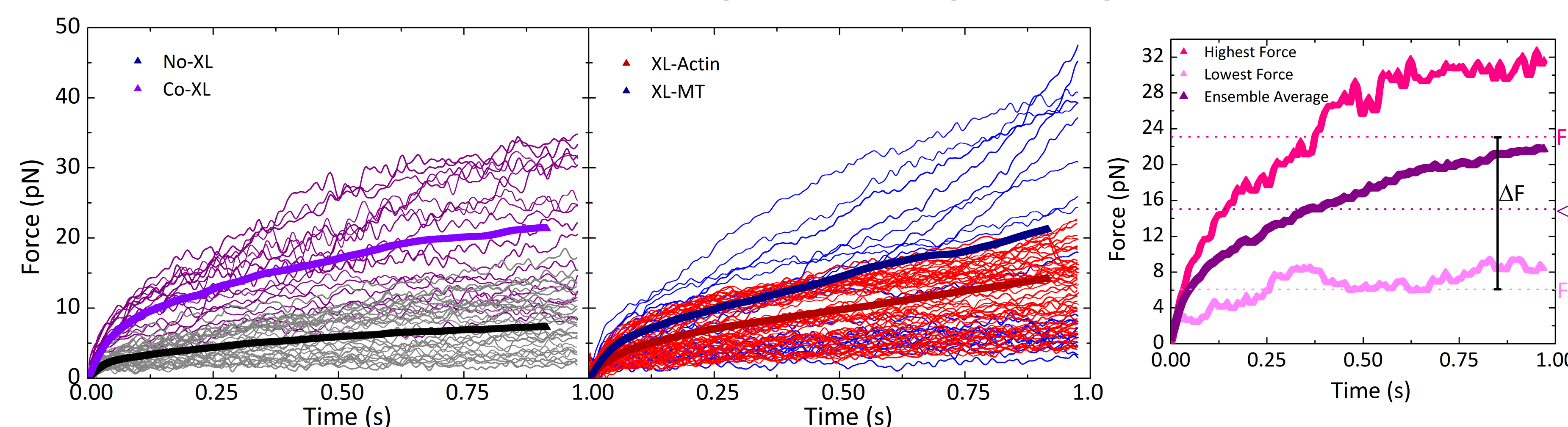


## Co-crosslinked networks are softer than single-crosslinked networks

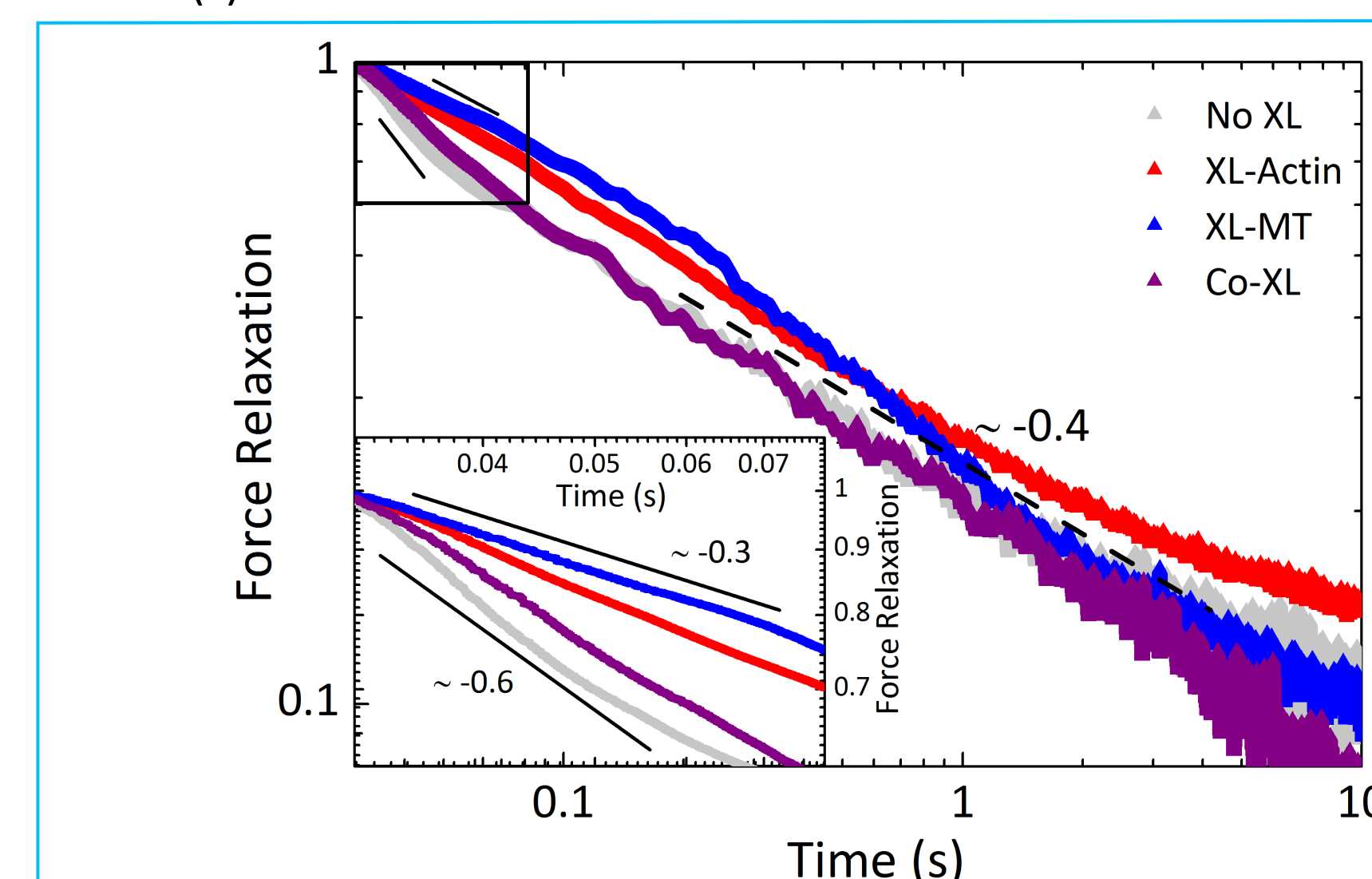
The differential modulus normalized by the starting point is plotted to quantify stiffness. Co-XL and No-XL display lower modulus values at early and late times indicating that they are more viscous compliant



## We characterize the microscale heterogeneities through the range in force curve trials



Co-crosslinking greatly suppresses heterogeneities in networks Co-XL networks are more homogenous.



Co-crosslinked networks exhibit faster initial force relaxation than single-crosslinked networks

**Conclusion:** Using dual color confocal microscopy and optical tweezers we find crosslinking motifs display different stiffnesses and mobility. Networks that are co-cross-linked are softer and more mobile than single cross-linked networks as the mobility of the microtubules is suppressed.

- References**
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