The effect of cytoskeletal crowding on the Mobility and Conformational Dynamics of Circular and Linear DNA

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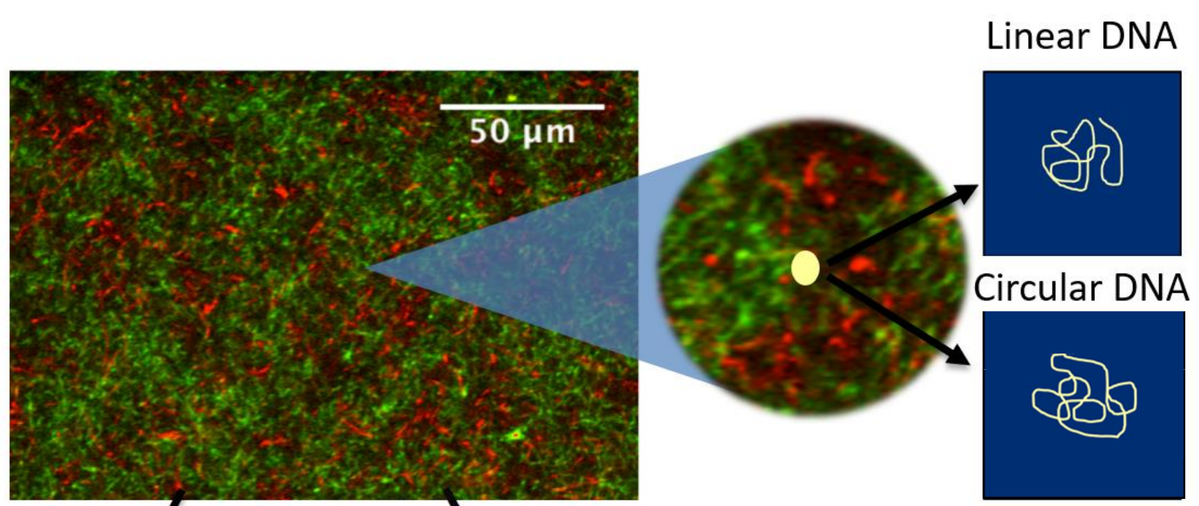
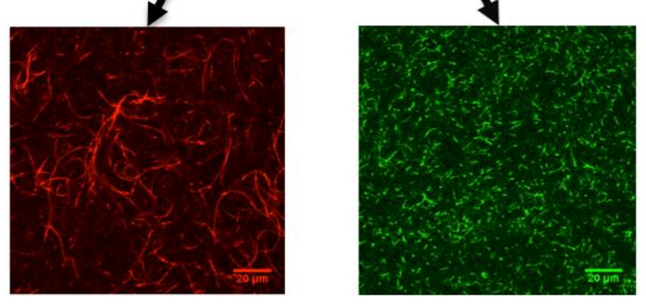
We characterize DNA dynamics when crowded by cytoskeletal protein networks

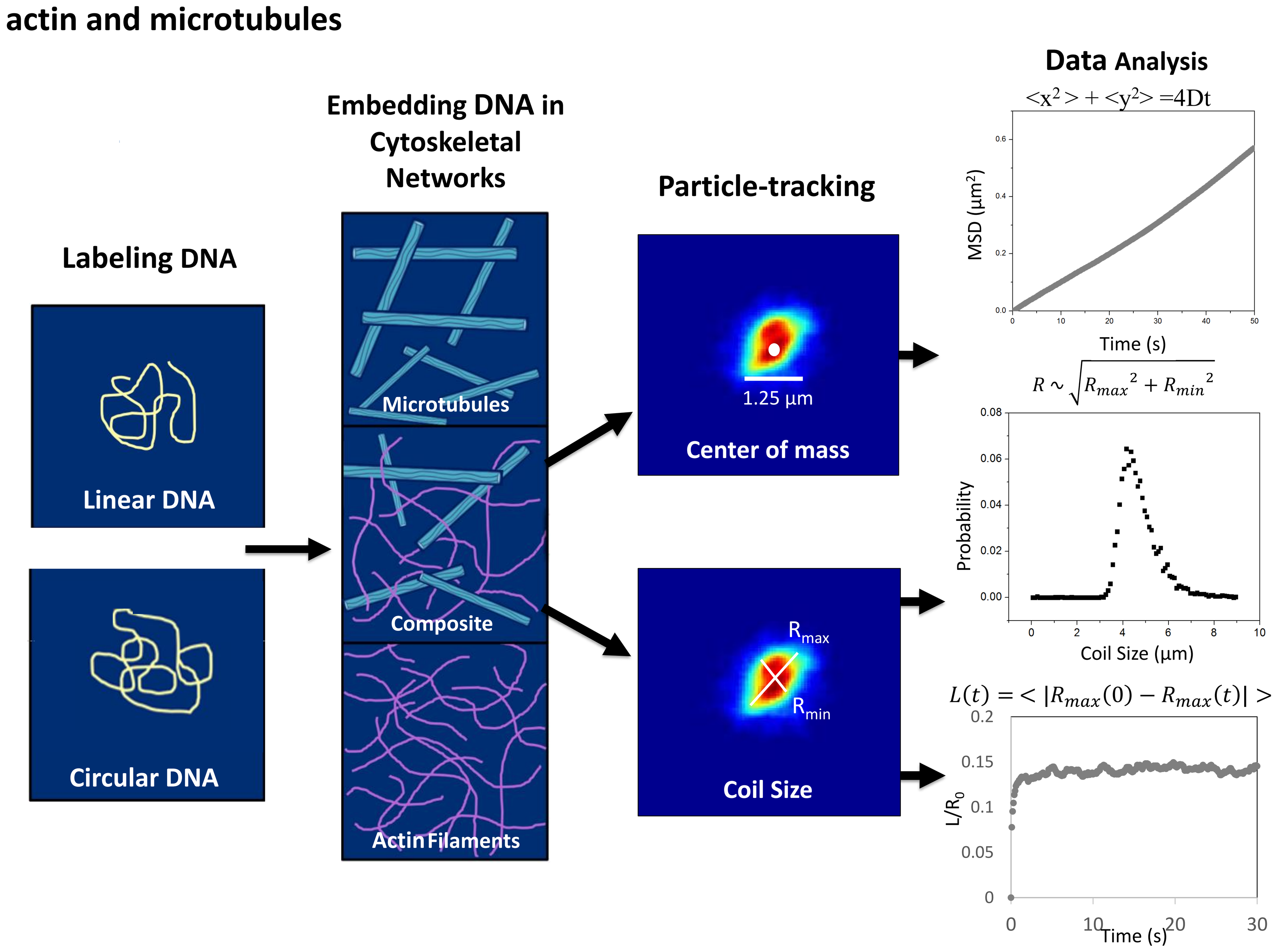




We measure the effect that crowding by cytoskeletal networks has on the diffusion of linear and circular DNA

Within the cell, diffusion of DNA through the cytoskeleton is necessary for many important processes such as drug delivery and suppressing viral transfection. The cytoskeleton is comprised of semiflexible actin filaments and rigid microtubules. However, how each filament affects the diffusion of DNA through the cytoskeleton remains unknown. Further, while DNA naturally exists in both linear and circular forms, how the DNA topology impacts its diffusion through the cytoskeleton is also not known.

We track single linear and circular DNA molecules diffusing through networks of actin and microtubules



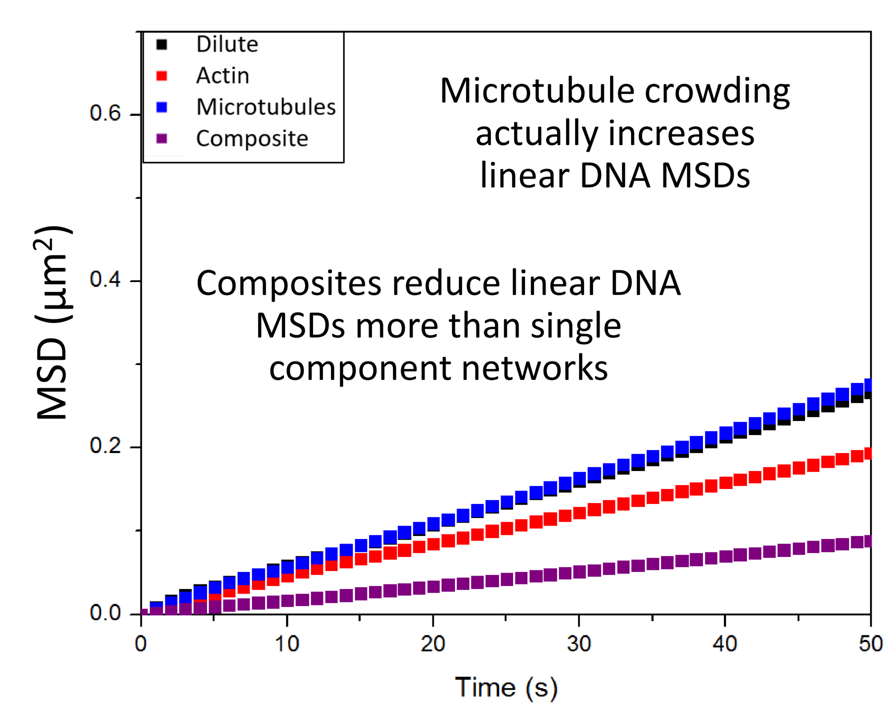
115 kbp linear or circular DNA is labeled with fluorescent dye YOYO-1 at a 1:5 dye:basepair ratio.

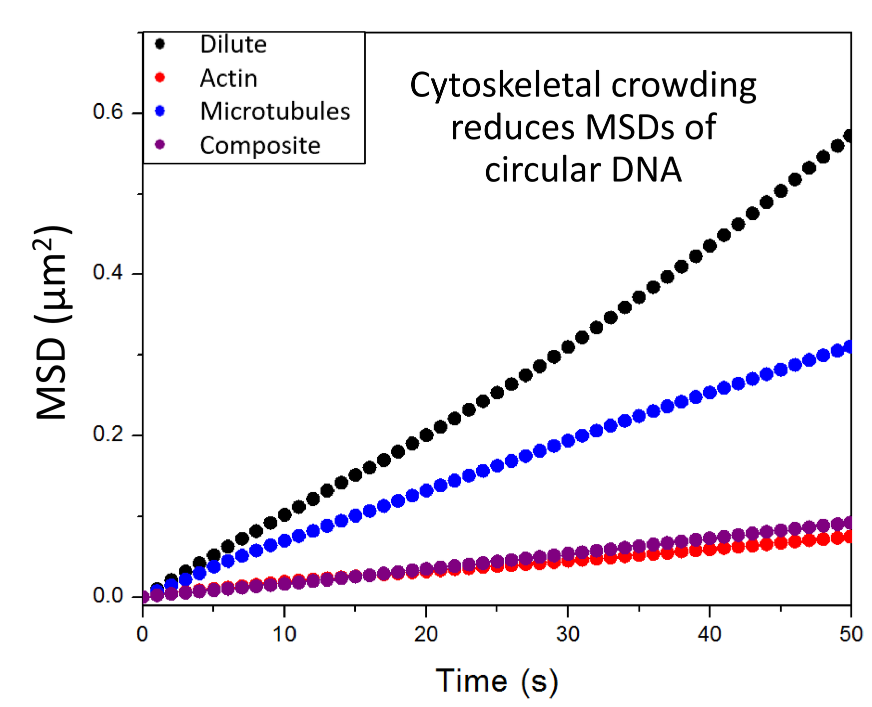
DNA is added to 11.6 µM actin monomers and/or tubulin dimers. Polymerization of actin and/or microtubules is achieved by adding 10 mM ATP and/or GTP and incubating at 37C for 30 mins.

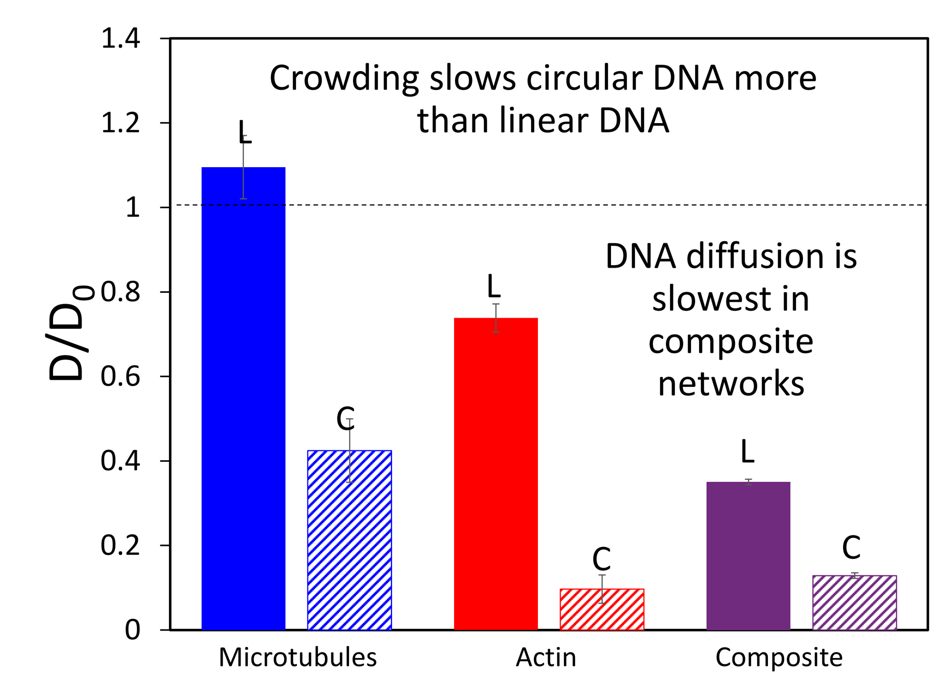
Single DNA molecules are imaged and tracked with an epifluorescence microscope with a 60X objective. We record 30 sec videos at 10 frames per second. >50 molecules are tracked for each condition.

The center-of-mass MSDs in x and y directions determine the diffusion coefficients (D). The lengths of the major and minor axes of the DNA measure the effective coil size (R) and the length-scale (L_{SS}) and rate of conformational fluctuations. (β).

Cytoskeletal networks slow the diffusion of circular DNA more than linear DNA

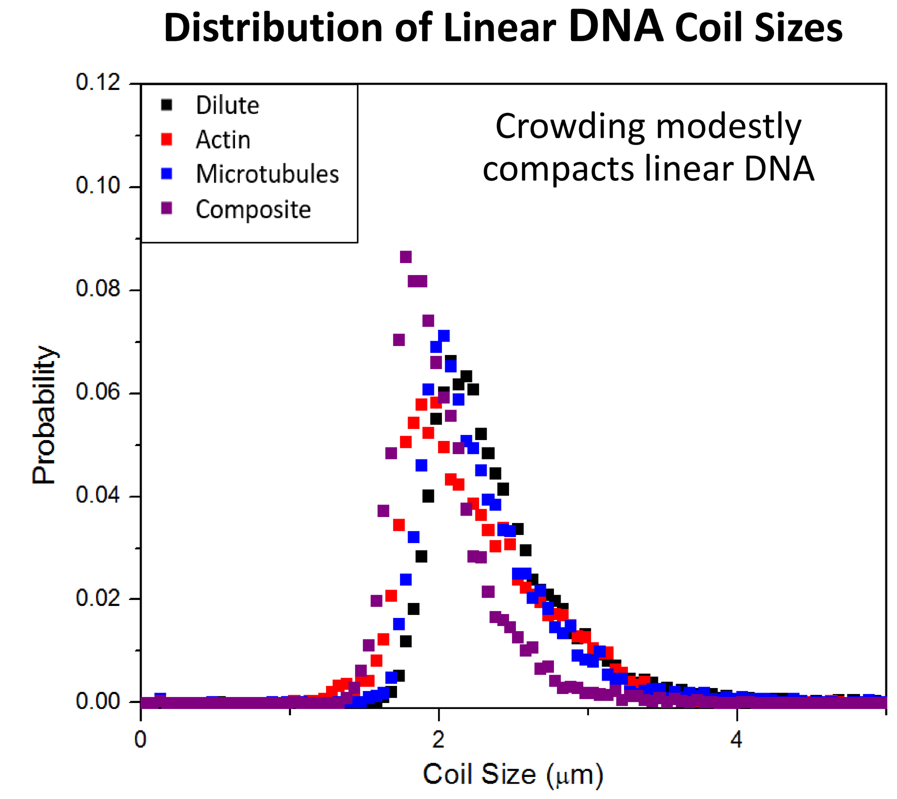


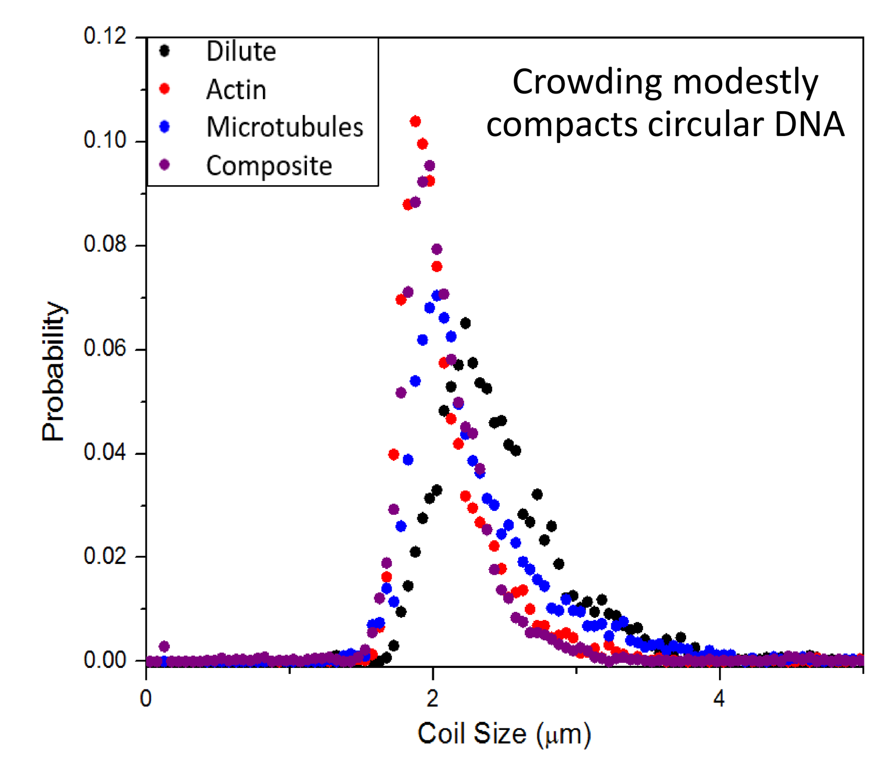


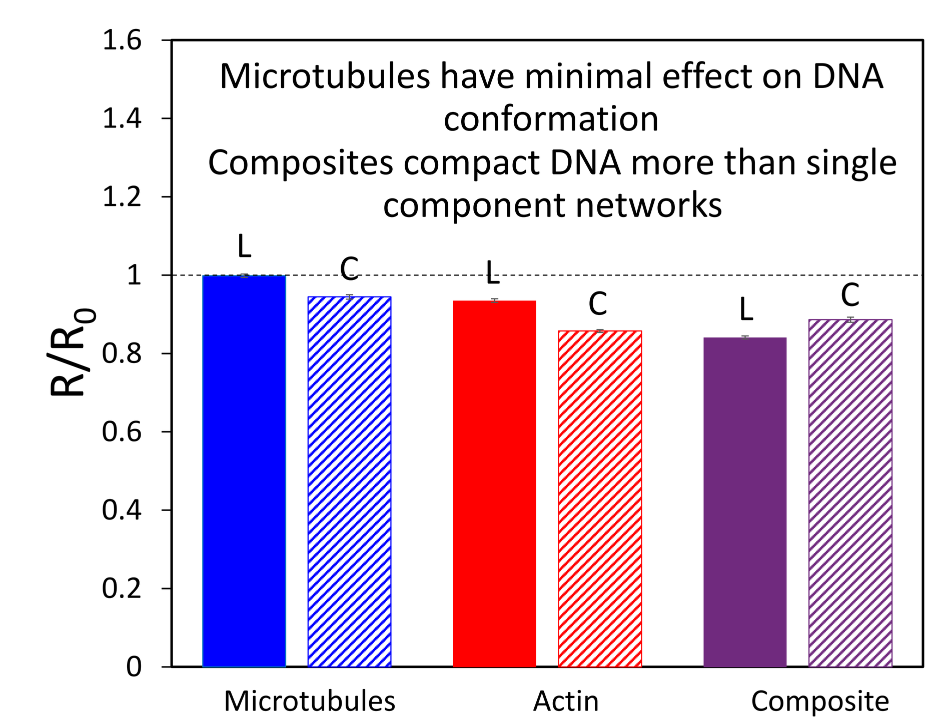


Both actin and microtubules together are needed to drastically slow both linear and circular DNA diffusion

Cytoskeleton crowding compacts both linear and circular DNA

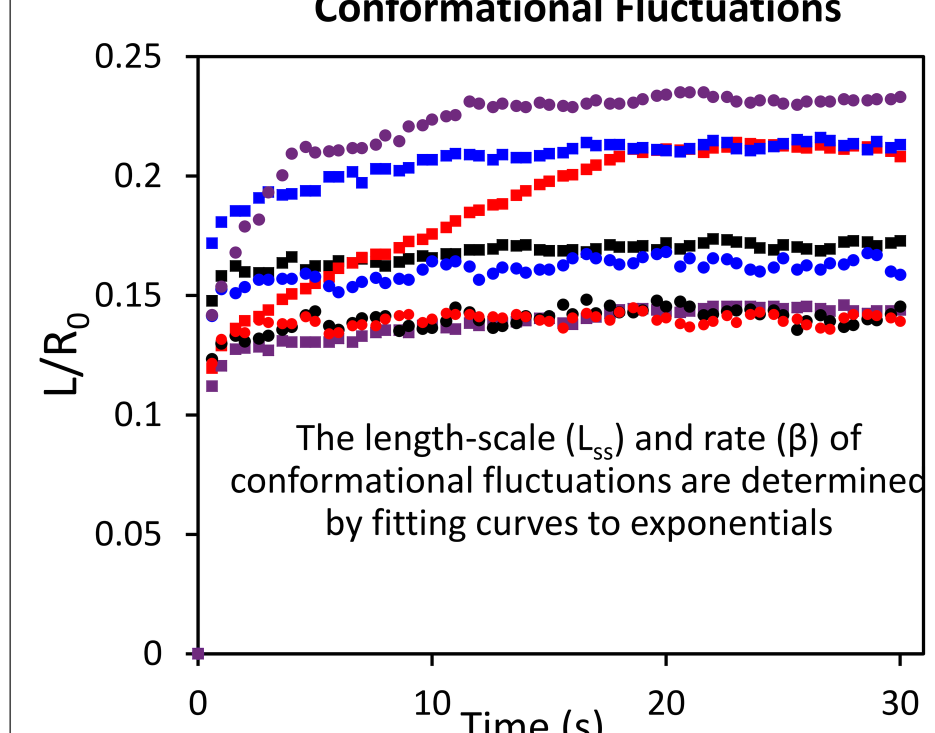


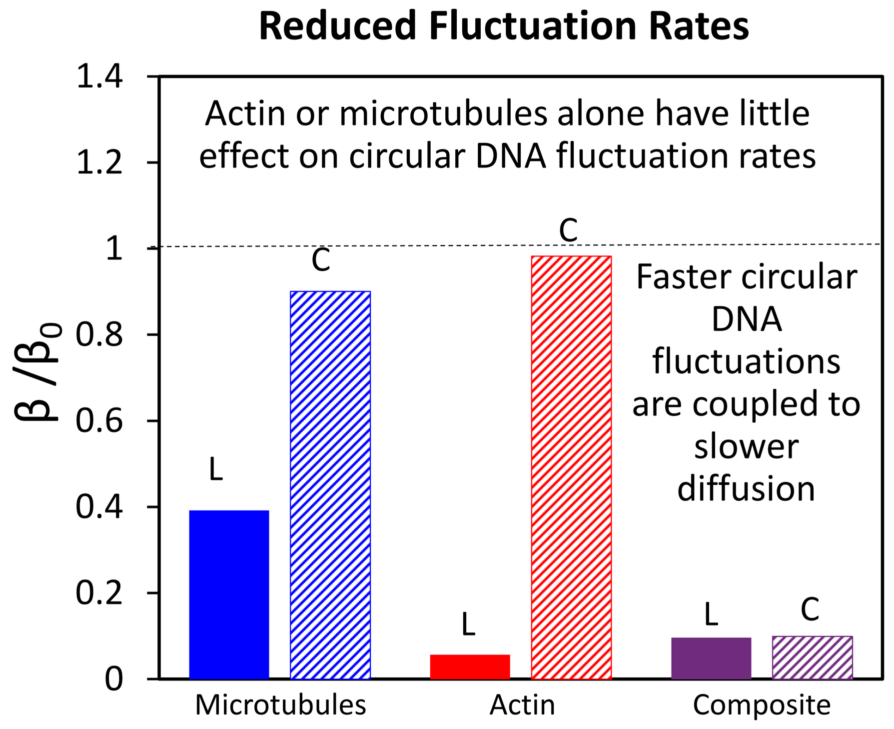


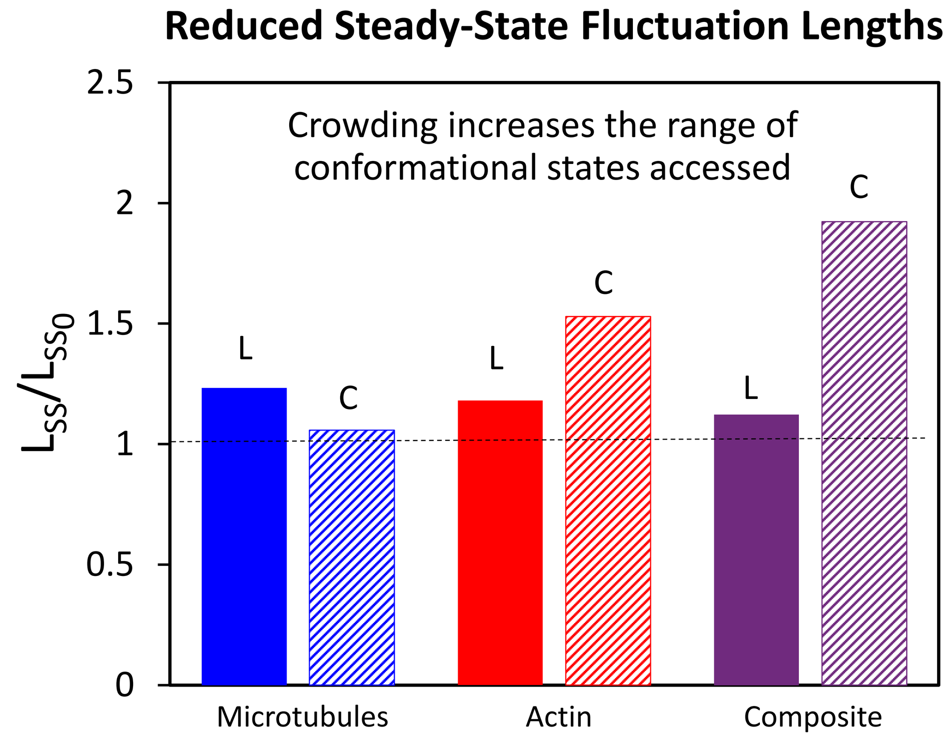


Composite networks compact DNA more than actin or microtubules alone

Cytoskeleton crowding *slows* the rates that DNA fluctuate between conformational states but *increases* the range of accessible states







Interactions between actin and microtubules are needed to appreciably slow the fluctuation rates of both linear and circular DNA

References

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2. R. M. Robertson-Anderson. 2007. Single Molecule Studies of DNA Dynamics and Intermolecular Forces.
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