We characterize DNA dynamics when crowded by cytoskeletal protein networks. 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.

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

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

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

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 37°C for 30 mins.

Single DNA molecules are imaged and tracked with an epifluorescence microscope with a GDX 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 are calculated by fitting curves to exponentials.

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

Cytoskeleton crowding compacts both linear and circular DNA.

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

Cytoskeleton crowding slowly affects DNA fluctuation rates but increases the range of accessible states.

Reduced Diffusion Coefficients

Reduced DNA Coil Size

Reduced Steady-State Fluctuation Lengths