DNA Transport through the Cytoskeleton

The shape and structure of biological cells is maintained in part by a cytoskeleton composed primarily of thick, rigid microtubules (L = 1 mm) made up of tubulin dimers, and thinner, more flexible actin filaments (F-actin, L = 17 μm) comprised of actin monomers. These proteins exist in both monomer and polymer form, depending on the requirements of the cell, and contribute to cellular crowding. As such, the cytoskeleton greatly influences the diffusion and conformational changes of nucleic acids and proteins in the cytoplasm.

We track single DNA molecules diffusing in varying cytoskeleton environments

We label 115 kbp linear DNA with YOYO-1 and image single DNA molecules using widefield fluorescence microscopy.

We construct cytoskeleton networks with varying degrees of rigidity

- Actin monomers (~5 nm)
- Tubulin Monomers (~100 nm)
- Actin Filaments (~10 μm x 10 nm)
- Microtubules (~10 μm x 25 nm)

We track single DNA molecules diffusing in each network.

Polymers compact DNA more than monomers due to smaller molecular spacing (actin) leads to crowding effects.

Dilute DNA exhibits normal diffusion → MSD = t1/2.

Cytoskeleton networks exhibit subdiffusive scaling: MSD = t^α with α < 1.

Slope of MSDs determines diffusion coefficients (MSD = 2Dt).

Network Rigidity and Mesh Size determine DNA mobility

- Polymers reduce mobility more than monomers.
- Smaller molecular spacing (actin) leads to slower diffusion.
- Actin filaments hinder DNA diffusion the most → reduced to ~50% of diffusion in microtubules.

Reduced DNA diffusion is coupled to coil compaction

- Probability distributions of DNA coil diameters show degree of coil compaction from dilute case.
- Polymers compact DNA more than monomers due to caging effects and depletion interactions.

Actin compacts DNA more than microtubules

- Actin monomers and polymers have smaller molecular spacing than microtubules.
- Smaller spacing leads to more compaction and slower diffusion.

Conclusion:

- Crowding by actin and tubulin induces compaction and decreased mobility of diffusing linear DNA.
- Crowding effects are increased with polymerization of cytoskeletal protein networks.
- Actin networks exhibit most dramatic crowding effects, due to their decreased molecular spacing.