# Diffusion and Conformational Dynamics of Single DNA Molecules Crowded by Cytoskeletal Proteins

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**Abstract.** The high concentrations of proteins crowding cells greatly influence intracellular DNA dynamics. These crowders, ranging from small mobile proteins to large cytoskeletal filaments such as semiflexible actin and rigid microtubules, can hinder diffusion and induce conformational changes in DNA. While previous studies have mainly focused on the effect of small mobile crowders on DNA transport, we examine the impact of crowding by actin filaments and microtubules. Further, because actin filaments and microtubules are formed by polymerization of actin monomers and tubulin dimers, respectively, we also investigate the role that the polymerization state of each protein plays in DNA transport and in the time-varying conformational changes of single DNA molecules diffusing in *in vitro* networks of polymerized and monomeric actin and tubulin. We find that crowding by actin monomers slows DNA diffusion while tubulin crowding actually increases diffusion coefficients. Monomeric actin crowding DNA diffusion, more than when actin is polymerized, while crowding by tubulin dimers increases DNA diffusion more than when tubulin is polymerized (microtubules). Further, we find unexpected relationships between DNA coil size and diffusion when crowded. All crowding conditions lead to some degree of DNA compaction, but less compaction enables faster dynamics.

**Keywords:** DNA dynamics, Single-molecule Particle Tracking, Polymer Dynamics, Cytoskeletal Crowding
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## INTRODUCTION

The biological cell is a highly crowded environment comprised of a wide variety of molecules that effectively crowd a molecule and prevent random intracellular movement.[1] The proteins that comprise the cytoskeleton are among the most important of these crowding macromolecules. The cytoskeleton, which supports cell shape, structure, and mobility, is composed primarily of thick, rigid microtubules (~10 μm x 25 nm), polymerized from tubulin dimers (10 nm), as well as thinner, semiflexible actin filaments (~10 μm x 10 nm) comprised of globular actin monomers (~5 nm).[2,3] These proteins can greatly influence the mobility of nucleic acids as they traverse the cytoplasm and can induce conformational changes that impact that stability of DNA.[1]

Indeed, cytoskeletal crowding has been identified as a key barrier to cytoplasmic transport of DNA[3,4] and influences important biological processes, including replication and transcription as well as gene expression and delivery.[1-6] Though research has been done on how to introduce DNA into a target cell for gene therapy[7], little is known of how cytoskeleton crowding impacts the DNA dynamics and conformational stability needed for efficient gene delivery.

Here, we investigate the diffusion and conformational dynamics of DNA crowded by the cytoskeletal proteins actin and tubulin. We track single DNA molecules diffusing in varying crowded solutions of actin and tubulin, in both monomeric and polymerized states. We find that cytoskeleton crowding compacts DNA and plays a complex role in DNA transport. Specifically, actin monomers slow DNA

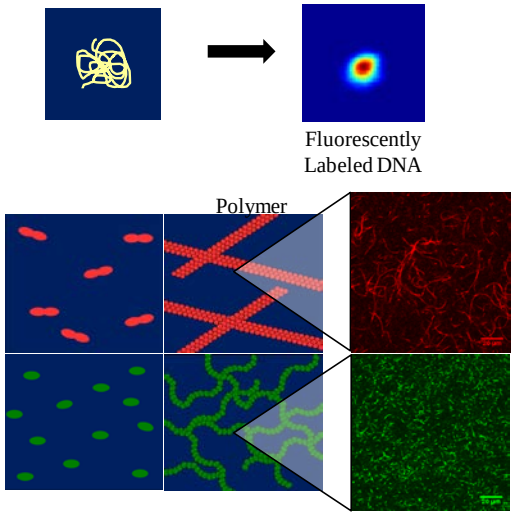


**FIGURE 1.** Experimental Schematic. (A) DNA molecules assume a random coil configuration in solution. When labeled with fluorescent dye, higher intensities denote higher mass density. (B) Cytoskeletal proteins exist in either monomer or polymer form. Polymerized proteins exhibit unique structural composition, as evident from confocal microscope images.

diffusion while tubulin aids DNA transport. Further, monomeric actin hinders DNA diffusion more than polymerized actin, while tubulin dimers increase DNA diffusion more than microtubules.

## METHODS & MATERIALS

Double-stranded linear 115 kbp DNA molecules are prepared through replication of bacterial artificial chromosomes (BACs) in *Escherichia coli*, following established protocols.[8,9] DNA molecules are then labeled with YOYO-I dye (Invitrogen) at a 4:1 ratio of base pairs to dye molecules (Figure 1A). 0.5 ng/μl of labeled DNA is added to 11.4 μM solutions of either rabbit skeletal actin or porcine brain tubulin (Cytoskeleton) suspended in 100 mM PIPES, 2 mM $MgCl_2$, and 2 mM EGTA (Figure 1B).[3] 0.05% Tween, 4% β-mercaptoethanol, 0.43 μg/μl glucose, and 72 ng/μl glucose oxidase are added to prevent surface interactions and photobleaching. Solutions are pipetted using a wide-bore pipette tip into a flow chamber consisting of a glass slide and coverslip separated by ~100 μm of double sided tape to accommodate ~15 μl of solution. To polymerize cytoskeleton proteins, 2 mM ATP (actin) or GTP (tubulin) is also added, and samples are incubated at 37 ºC for 30 minutes.

To measure the transport and conformations of diffusing DNA molecules, we image single diffusing DNA molecules for 30 seconds at 10 frames per second using a 60x objective and high-speed CCD camera on a Nikon A1R Epifluorescence microscope. We track >50 molecules for each condition. We measure and track the center of mass (COM) position as well as the lengths of the major and minor axes ($R_{max}$, $R_{min}$) of each molecule in each frame using custom-written software (Matlab) (Figure 2).[10]

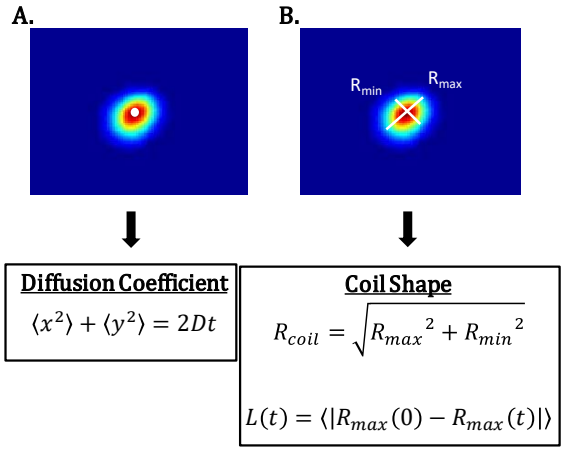


**FIGURE 2.** Measurements of interest. (A) Center-of-mass is tracked through center-of-intensity in order to calculate diffusion coefficients through mean-squared displacements. (B) Major and minor axis lengths are tracked in time *t* to quantify a conformational size and fluctuation length via the displayed equations.

We calculate the COM mean-squared displacement in the *x* and *y* directions ($\langle\Delta x^2\rangle$, $\langle\Delta y^2\rangle$) to determine the diffusion coefficient *D* via $\langle\Delta x^2\rangle + \langle\Delta y^2\rangle = 2Dt$. Error bars are calculated using the bootstrap method for 1000 sub-ensembles. We quantify the conformational size of the DNA ($R_{coil}$) from the major and minor axis length measurements via $R_{coil} = (R_{max}^2 + R_{min}^2)^{1/2}$. Finally, we characterize the time-dependence and length scales of conformational fluctuations by examining the extent to which $R_{max}$ varies from its initial value over time. Specifically, we define a fluctuation length *L* calculated as $L(t) = \langle|R_{\max}(0) - R_{max}(t)|\rangle$. The time over which this quantity reaches a steady-state value can be understood as the rate of conformational fluctuations. The steady-state length scale reached can be understood as the length scale over which a molecule fluctuates, or the range of different conformational states it accesses.

## RESULTS

### Actin Crowding

We first examine the diffusion coefficients of DNA molecules crowded by monomeric or polymerized actin compared to the case of no crowding. Crowding by actin inhibits DNA diffusion, with monomers slowing diffusion more than filaments. Normalizing by the dilute diffusion coefficient reveals a ~50% and ~25% decrease in diffusion coefficients when crowded by actin monomers or filaments, respectively (Figure 3A) Reduction in diffusion coefficients is coupled with a modest decrease in average coil size, from $R_{coil}$ = 2.175 μm with no crowding to $R_{coil}$ = 1.925 μm and $R_{coil}$ = 1.875 μm in actin monomers and filaments, respectively (Figure 3A).

Figure 3B shows the reduced length scales as well as reduced rates at which conformational fluctuations are taking place: a higher length scale here denotes more conformational states being accessed. Conformational fluctuation rates are greatly hindered by the presence of actin, regardless of polymerization state. Molecular conformational states fluctuate on larger length scales at significantly slower rates (Figure 3B), accessing more conformational states over time.

### Tubulin Crowding

Figure 3A shows that crowding by either tubulin dimers or microtubules speeds up DNA diffusion. While crowding by microtubules results in only a 4% increase in diffusion coefficient compared to the case without crowders, tubulin dimers induce a 47% increase in DNA diffusion coefficients. Despite this large

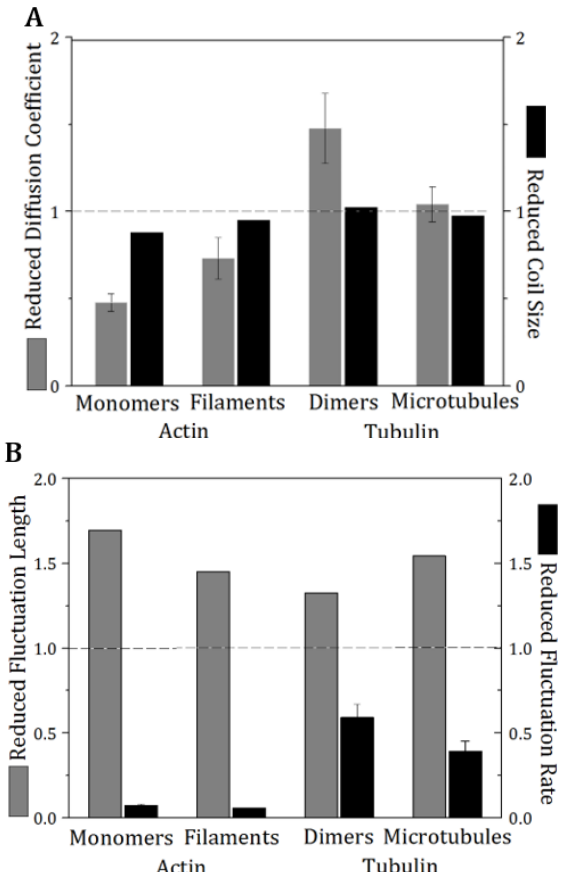


**FIGURE 3.** DNA transport and conformational dynamics when crowded by cytoskeleton proteins. (A) Tracking COM mean-squared displacement results in DNA diffusion coefficients (grey) Tracking of major and minor axis lengths results in average DNA coil sizes (black). Both quantities are normalized (reduced) by the corresponding value with no crowding (dotted line). Results show less compaction leads to faster dynamics. (B) Steady-state conformational fluctuation lengths (grey) and fluctuation rates (black) reduced by the corresponding value with no crowding (dotted line). Results show crowding increases the number of accessed conformational states, but at timescales much lower than without crowding.

increase in diffusion coefficient, there is no evident change in coil size for either case (Figure 3A).

However, as shown in Figure 3B, both tubulin and microtubule crowding allow DNA molecules to access a wider range of conformational states compared to the case of no crowding. While DNA fluctuates over large length scales, the rate of fluctuations is reduced by a factor of ~2 in each case.

## DISCUSSION

Crowding by actin reduces DNA diffusion, following expected crowded behavior. Actin filaments suppress conformational fluctuations of DNA more than actin monomers, possibly enabling DNA to undergo faster COM diffusion when crowded by the actin filaments compared to monomers.

However, crowding by tubulin enhances DNA diffusion, with tubulin dimers inducing significantly faster DNA diffusion microtubules. This increase in diffusion coefficient runs counter to most accepted crowding models, since diffusion is quicker than in even the dilute condition. Furthermore, quicker diffusion rates $D$ are normally coupled with smaller coil sizes $R_{coil}$, as described by the Stokes-Einstein diffusion relation between viscosity η, thermal energy $k_BT$, and molecule radius $r$

$$D = \frac{k_B T}{6\pi\eta r}$$

Overall, we find that less compaction leads to faster dynamics and that all cytoskeletal crowding leads to slower conformational changes but access to a broader range of conformational states.

## CONCLUSION

We investigate the role of cytoskeleton crowding on the diffusion and conformational dynamics of DNA molecules. We show that actin and microtubules have highly different effects on DNA diffusion, with actin slowing DNA transport while tubulin surprisingly speeds up diffusive transport. Further, while crowding by polymerized actin filaments hinders DNA diffusion less than when actin is monomeric, we find the opposite effect with tubulin. Namely, crowding by tubulin dimers increases DNA diffusion more than polymerized microtubules. Unlike the impact on DNA diffusion, crowding by all cytoskeleton proteins has similar effects on DNA conformational dynamics. All crowding conditions induced modest DNA compaction, slower conformational fluctuation rates, and a wider range of conformational states accessed.

Future work will examine how crowding by composite systems of both actin and microtubules impacts DNA dynamics and the role that DNA topology plays in crowding-induced dynamics.

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## REFERENCES


1. R. J. Ellis, *Current Opinion in Structural Biology* **11**, 114 (2001).
2. F. Gittes, B. Mickey, J. Nettleton, and J. Howard, *The Journal of Cell Biology* **120**, 923-934 (1993).
3. S. N. Ricketts, J.L. Ross, and R.M. Robertson-Anderson, Preprint: *BioRxiv http://doi.org/10.1101/262089* (2018).
4. E. Dauty and A.S.Verkman, *Journal of Biological Chemistry* **280,** 7823 (2005).
5. H. Matsuda, G.G. Putzel, V. Backman, and I. Szleifer, *Biophysical Journal* **106**, 1801 (2014).
6. J. Pelletier, K. Halvorsen, B.-Y. Ha, R. Paparcone, S. J. Sandler, C. L. Woldringh, W. P. Wong, and S. Jun, *Proceedings of the National Academy of Sciences* **109,** E2649 (2012).
7. S. Mali, *Indian Journal of Human Genetics* **19**, 3 (2013).
8. S. Laib, R. M. Robertson-Anderson, and D.E.S. Smith, *Macromolecules* **39**, 4115 (2006).
9. S. M. Gorczyca, C. D. Chapman, and R.M. Robertson-Anderson, *Soft Matter* **11,** 7762 (2015).
10. C.D. Chapman, S.M Gorczyca, and R.M. Robertson-Anderson, *Biophysical Journal* **108,** 1220 (2015).