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Citation: **1518**, 517 (2013); doi: 10.1063/1.4794627
View online: http://dx.doi.org/10.1063/1.4794627
View Table of Contents: http://aip.scitation.org/toc/apc/1518/1
Published by the American Institute of Physics

# Active DNA Gels

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**Abstract.** Research into the mechanics and fluctuations of living cells has revealed the key role played by the cytoskeleton, a gel of stiff filaments driven out of equilibrium by force-generating motor proteins. Inspired by the extraordinary mechanical functions that the cytoskeleton imparts to the cell, we sought to create an artificial gel with similar characteristics. We identified DNA, and DNA-based motor proteins, as functional counterparts to the constituents of the cytoskeleton. We used DNA self-assembly to create a gel, and characterized its fluctuations and mechanics both before and after activation by the motor. We found that certain aspects of the DNA gel quantitatively match those of cytoskeletal networks, indicating the universal features of motor-driven, non-equilibrium networks.

**Keywords:** active soft matter; motor proteins
**PACS:** 87.15.La

## INTRODUCTION

The mechanics of eukaryotic cells are largely defined by the cytoskeleton, a crosslinked network of proteinaceous filaments (notably actin and microtubules) that are acted upon by energy-consuming motor proteins of the myosin and kinesin families. Motor-generated forces propagate throughout the network and assist a variety of cellular functions, including shape changes, cell division, and motility.

The relative simplicity of the cytoskeleton, and its partial autonomy from genetic control, raises the hypothesis that cytoskeletal activities can be replicated by an *artificial* system. Such a system would be useful to investigate issues of non-equilibrium soft matter, and could eventually find technological utility.

To create an artificial active gel, we need two components: structural filaments that can be crosslinked to form a gel of finite stiffness, and motors that transduce chemical energy into mechanical work. The choice of motor is more difficult– purely synthetic motors exist, but are relatively limited in comparison to highly-evolved biological motors. One large class of motors that can be exploited are those that work on DNA: the biological needs to replicate, segregate and repair genomes have led to the evolution of a large range of DNA-based motor proteins. Further, DNA itself is an excellent choice for a filament, as it is highly water-soluble, and permits precise control of structure through manipulation of its base sequence.

Here, we discuss the design, synthesis, and characterization of a gel of DNA that is acted upon by a DNA-based motor, FtsK. We find that motor activity stiffens the gel, and creates active fluctuations whose spectral and temporal signature match those found in cytoskeletal studies [1, 2, 3, 4], and predicted theoretically [5, 6]. Our work thus points out universal features of motor-driven gels. We note that aspects of this work are discussed more fully in Ref. [7].

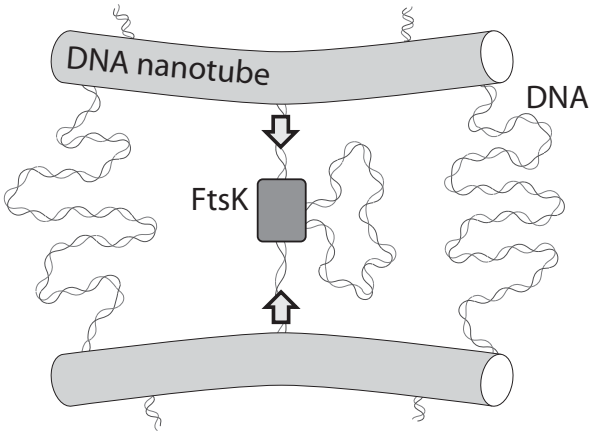


**FIGURE 1.** Schematic of active DNA gel design. Stiff DNA nanotubes are crosslinked by long double-stranded DNA linkers. The motor protein FtsK binds and contracts the linkers, leading stiffening and fluctuations throughout the gel.

*4th International Symposium on Slow Dynamics in Complex Systems*
AIP Conf. Proc. 1518, 517-519 (2013); doi: 10.1063/1.4794627

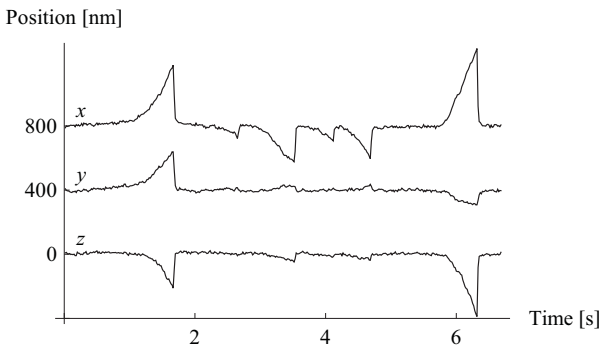


**FIGURE 2.** Three-dimensional trajectory of a bead attached to the top of a DNA gel in the active state. Traces are shifted for clarity.

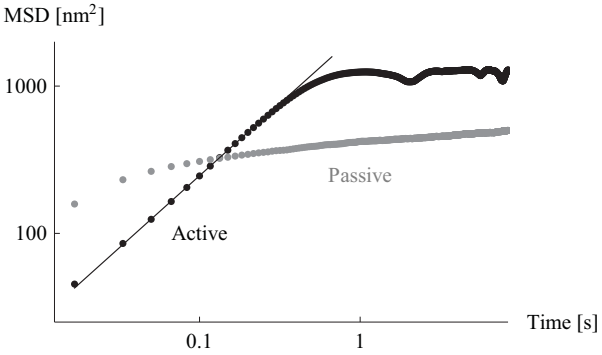


**FIGURE 3.** Mean-squared displacement (MSD) vs. time calculated from the $z$ axis trajectory of a bead attached to a DNA gel, before ('passive') and after ('active') activation by addition of a motor protein. The active data is fit to a power-law at short times; the best-fit curve is plotted, and has exponent 0.98.

## GEL DESIGN

To create a DNA-based active gel requires synthesizing a crosslinked network that retains the ability to host active motor proteins. As most motor proteins act on relatively long tracts of double- or single-stranded DNA, such filaments must be included. However, the rapidly fluctuating nature of flexible DNA makes microscopic characterization of such filaments difficult. Thus, we formulated a design in which highly-stiff DNA nanotubes are crosslinked by long, double-stranded DNA linkers. The nanotubes are self-assembled from DNA oligos, and are effectively bundles of 10–12 double-stranded DNA molecules, stabilized by multiple crossover junctions [8]. Their stiffness is characterized by a persistence length of several microns, compared to the $\approx 50$ nm persistence length of double-stranded DNA.

While multiple motors exist that act on DNA, we sought to include a motor that directly mimicked the active contractile capabilities of myosin. FtsK50C, a motor construct derived from the bacterial protein FtsK, fit this criteria: single-molecule studies have shown that FtsK50C forms motor aggregates that, in the presence of ATP, have a strong and fast contractile activity on single filaments of DNA [9].

A schematic of our final gel system is shown in Fig. 1. Gel synthesis was achieved by first self-assembling nanotubes, than crosslinking them with long , plasmid-derived double-stranded DNA linkers. Typical final gel dimensions were mesoscopic, usually a few microns on a side.

## RESULTS AND DISCUSSION

To measure the gel properties, we attached gels to a glass surface, and attached micron-scale beads to the tops of the gels. We then visualized the beads in a light microscope, and tracked their position over time at 60 Hz. In the absence of the motor, the bead trajectories displayed stable, gaussian-distributed Brownian trajectories [7]. However, upon addition of motor and ATP, the trajectories displayed large, sawtooth excursions (Fig. 2). We interpret these excursions as being due to contraction-and-release events by single FtsK50C motors. This conclusion is justified by the identical sawtooth shape seen in measurements of the length of single DNA chains upon FtsK50C contraction [9]. In the gel, excursions happened in all three dimensions, indicating motors were binding and contracting in random spatial positions throughout the gel.

To quantify bead fluctuations, we calculated the mean-square displacement (MSD) of bead position vs. lag time (Fig. 3). Before motor activation, the MSD showed a rise at short lag times, followed quickly by a plateau. This behavior is exactly as expected for a particle moving in a harmonic potential (here, caused by tethering to the elastic gel) and subject to thermal fluctuations. In contrast, in the presence of the motors, the crossover to the plateau moved to longer lag times, and the short-lag-time behavior indicated a nearly linear increase of MSD with time (as in free diffusion). The shift of the crossover is surprising, as other analyses indicated that the DNA gel *stiffened* upon motor activation– if the only effect of motors were gel stiffening, we would expect the crossover to move to shorter times, contrary to observation.

The emergence of a long diffusive-like regime in the active gel agrees with theoretical predictions [5, 6] that focus on the sudden release of the substrate by the contractile motor. The sudden release causes a jump in measured bead position, and thus a step-like trajectory. The Fourier transform of a step function has a $1/f$ spectral signature, and thus a $1/f^2$ power spectrum. Significantly, this matches the $1/f^2$ power spectrum of a freely-

diffusing particle, thus explaining why a linear MSD with time was observed for a tethered particle subject to active, non-equilibrium fluctuations. It is important to recognize that free diffusion, and active gel fluctuations, display similar dynamics for very different reasons: in free diffusion, the driving force (Langevin fluctuations) are white, but the response (viscous drag) carries a $1/f$ dependence. In the active gel, it is the driving force that carries a $1/f$ dependence, while the response (gel elasticity) is roughly white.

A full understanding of the dynamics of the DNA-based active gel requires an estimate of the gel's viscoelastic properties. Acquiring such an estimate through microrheology requires separating thermal and motor-driven fluctuations, which can be approximately achieved through the analysis of van Hove distributions [7]. However, as shown by Mizuno *et al.* [1], precise quantification of an active gel's viscoelastic properties requires the combination of passive microrheological methods (as used here) with active microrheology, where the bead is measured as it responds to an external force arising from, for example, an optical trap or magnetic field. The gels described here are too small (only a few microns on a side) to permit active microrheological measurement; thus, a future direction of this work is to synthesize large DNA-based active gels that permit precise mechanical characterization through active microrheology.

## CONCLUSION

We have discussed the design and characterization of a DNA-based gel that displays non-equilibrium, quasi-diffusive dynamics upon activation with a motor protein. These observations are quantitatively similar to *in vivo* and *in vitro* measurements of cytoskeletal systems [1, 2, 3, 4], thus indicating we have successfully created an artificial active system whose mechanics match those of the cytoskeleton. Our results underline the key role played by sudden unbinding of the motor in defining gel dynamics, in agreement with theory [5, 6]. Finally, our work demonstrates the universal internal mechanics of motor-driven gels, and supports the feasibility of creating artificial gels with cell-like mechanical functions.

## ACKNOWLEDGMENTS

We thank David Sherratt for providing protein. We acknowledge funding support from the UCSB International Center for Materials Rearch, and the UCSB Materials Research Laboratory, an NSF-funded MRSEC (award No. DMR 1121053).

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