The complex rheology of active dynein-microtubule networks measured with optical tweezers

Mitchell Woodhouse¹, Kaila Craig², Ashok Pabbati³, Subash Godar³,⁴, Olga Kuksenok⁵, Joshua Alper³,⁴,⁶

¹Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA; ²Department of Chemistry and Physics, Fayetteville State University, Fayetteville, NC
³Department of Physics and Astronomy, Clemson University, Clemson, SC; ⁴Eukaryotic Pathogen Innovation Center, Clemson University, Clemson, SC; ⁵Department of Materials Science and Engineering, Clemson University, Clemson, SC; ⁶Department of Biological Sciences, Clemson University, Clemson, SC

ABSTRACT
Motile microstructures underlie a new class of active materials that have multiple potential medical applications. One such application is the development of an oscillatory active gel system, which will have dynamic and tunable properties for specific use cases. Motor proteins such as kinesin and cytoplasmic dynein have been used in conjunction with microtubules to create adaptable gels, however, the motion of these gels results in a clustering of proteins as the motors ‘walk’ down the microtubules. In comparison, axonemal dynein utilizes a ‘power stroke’ to move microtubules while the protein remains steady. To facilitate oscillatory motion, here we build an active network by crosslinking microtubules with axonemal dynein. We characterized this network using embedded microspheres driven with optical tweezers to measure the complex rheology of the system. We found that the use of microspheres with the optical tweezer allowed us to compare experimental viscosity with the known viscosity of water and 50% glycerol solutions, confirming the accuracy of this method. Furthermore, we found that the viscosity, loss modulus, and storage modulus of an active network varied throughout the network. Our results demonstrate that axonemal dynein-microtubule networks are similar in structural form to kinesin-microtubule gels while maintaining a unique rheology. Further investigation of this difference will allow future materials to utilize the natural oscillation of axonemal dynein.

METHODS
Measuring hydrolysis of dynein: Axonemal dynein utilizes the energy of ATP hydrolysis to glide along microtubules. Here we used the release of phosphate from this reaction as a detector of dynein activity, and back calculated the ATP consumption of dynein.

Using the optical trap: Microspheres of various sizes were suspended in media and trapped as seen on the left. The stage was oscillated while holding the trap constant, subjecting the bead to drag forces of the media. In the right image, the displacement of a bead stuck to the stage (top) and in the trap (bottom) are shown.

THEORY

\[ G'(\omega) = \frac{F_{\text{max}}}{\pi R_{\text{max}}} \cos \Delta \phi \]

\[ G''(\omega) = \frac{F_{\text{max}}}{\pi R_{\text{max}}} \sin \Delta \phi \]

Complex rheology equations: \( G'(\omega) \), the storage modulus characterizes elastic response. \( G''(\omega) \), the loss modulus, characterizes viscous response. The image on the right demonstrates bead displacement in media.

RESULTS

I. Turnover rate of Dynein

ATPase activity of axonemal dynein: Hydrolysis of ATP into ADP by dynein activity is visualized through an ENZcheck assay kit (left). Visual analysis demonstrates the dynein used is active, however, numerical analysis of the slope (right) returns a turnover rate of 33.6 ATP molecules/dynein/second, roughly 3x the known rate (1-10 ATP/Dynein/second).

II. Comparison of Relative Viscosity

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Comparison of bead displacement: The relative viscosity of a fluid can be determined by equating the drag force (\( 6\pi r \eta \omega \)) and the trapping force (\( k \delta \)). Assuming a constant stiffness throughout all media, the ratio of bead displacement over frequency (\( \delta / \omega \)) between media represents the relative viscosity (\( \eta \)) of each media. 50% glycerol has a higher relative viscosity than water, with 25% glycerol (not shown) between the 0% and 50% solutions.

REFERENCES
2. Robertson-Anderson, ACS Micro Letters 2018
3. Paul et al., J. Phys.: Condens. Matter 2018

CONCLUSION

- Phase shift and relative viscosity quantify viscoelasticity
- Dynein demonstrated to be active
- Data provides framework for analysis of active material

FUTURE DIRECTIONS

- Characterize the complex rheology of active dynein-microtubule systems
- Test different frequencies to verify theoretical modeling

ACKNOWLEDGEMENTS
This material is based upon work supported by the National Science Foundation under Grant No. 1757658