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14. ABSTRACT
Motor proteins such as myosin or kinesin play a major role in cellular cargo transport, muscle contraction, cell division, and also in engineered nanodevices. Quantifying the collective behavior of coupled motors is critical for our understanding of these systems. An excellent model system is the gliding motility assay, where hundreds of surface-adhered motors propel one cytoskeletal filament such as an actin filament or a microtubule. The filament motion can be observed using fluorescence microscopy, revealing fluctuations in gliding velocity. These velocity

15. SUBJECT TERMS
biomolecular motor, velocity fluctuation, kinesin, microtubule, motor protein, Brownian dynamics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Henry Hess
UU	UU	UU	UU		19b. TELEPHONE NUMBER 212-854-7749

Report Title

Final Report: Measurement of velocity fluctuations to infer attachment geometry and interactions of mechanically coupled molecular motors in one-dimensional arrays

ABSTRACT

Motor proteins such as myosin or kinesin play a major role in cellular cargo transport, muscle contraction, cell division, and also in engineered nanodevices. Quantifying the collective behavior of coupled motors is critical for our understanding of these systems. An excellent model system is the gliding motility assay, where hundreds of surface-adhered motors propel one cytoskeletal filament such as an actin filament or a microtubule. The filament motion can be observed using fluorescence microscopy, revealing fluctuations in gliding velocity. These velocity fluctuations have been previously quantified by a motional diffusion coefficient, which Sekimoto and Tawada explained as arising from the addition and removal of motors from the linear array of motors propelling the filament as it advances, assuming that different motors are not equally efficient in their force generation. A computational model of kinesin head diffusion and binding to the microtubule allowed us to quantify the heterogeneity of motor efficiency arising from the combination of anharmonic tail stiffness and varying attachment geometries assuming random motor locations on the surface and an absence of coordination between motors. Knowledge of the heterogeneity allows the calculation of the proportionality constant between the motional diffusion coefficient and the motor density. The calculated value (0.3) is within a standard error of our measurements of the motional diffusion coefficient on surfaces with varying motor densities calibrated by landing rate experiments. This allowed us to quantify the loss in efficiency of coupled molecular motors arising from heterogeneity in the attachment geometry.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

H. Hess: “Velocity fluctuations, alignment, and assembly in kinesin gliding motility assays”, Symposium “Multiscale Motility of Molecular Motors”, Max-Planck-Institute for Colloid Research Potsdam-Golm, Germany (9/25/2013) invited

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received		Paper
10/30/2015	1.00	Henri Palacci, Ofer Idan, Megan J. Armstrong, Ashutosh Agarwal, Takahiro Nitta, Henry Hess. Velocity Fluctuations in Kinesin-1 Gliding Motility Assays Originate in Motor Attachment Geometry Variations, Langmuir (10 2015)

TOTAL: **1**

Number of Manuscripts:

Books

Received	Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Ofer Idan	1.00	
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Henry Hess	0.11	
FTE Equivalent:	**0.11**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

Names of Personnel receiving masters degrees

NAME	
Total Number:	

Names of personnel receiving PHDs

NAME	
Ofer Idan	
Total Number:	**1**

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

I. Statement of the problem studied
Molecular motors have the potential to transform the conversion of chemical energy into mechanical work, with wide-ranging applications in sensing and actuation. A key challenge is force multiplication via coordinated arrays of molecular motors. Arrays of molecular motors exhibit rich dynamics, due to the stochasticity of the individual chemical reaction steps and the nonlinearity of the mechanical coupling between motors. A model system to study such a motor array is a kinesin-powered molecular shuttle, where dozens of surface-adhered kinesin motor proteins simultaneously bind to and propel a microtubule. The dynamics of the system can be characterized by the mean of the microtubule velocity and the fluctuations around it. The relative magnitude of the fluctuations is predicted to depend on the motor density and the attachment geometry. The objective of the project was to experimentally determine velocities and velocity fluctuations as a function of motor densities and draw inferences with respect to motor attachment geometry and motor interactions. It was hypothesized that the system dynamics undergoes a phase transition as the kinesin tail conformations change from the mushroom to the brush regime with increasing kinesin density.

II. Summary of the most important results

Through a computational model of kinesin head diffusion, we were able to estimate the distribution of kinesin tail extensions for kinesins uniformly distributed on a surface and bound to a microtubule. We then combined this distribution and the calculated anharmonic force- extension relation to quantify the theoretical heterogeneity of motor force production. Our model yields a value for the heterogeneity factor a of 0.3.
By combining measurements of the kinesin surface density and of the motional diffusion coefficient, we were also able, for the first time, to determine this heterogeneity factor experimentally. While Sekimoto and Tawada proposed that the constant is about one, in our assay a value of 0.56 ± 0.3 was found, in good agreement with our theoretical value.
Under our assumptions, we have shown that the variability in the displacement of the microtubule after each motor step can be explained by the variable force contribution of each motor. In our model, each motor has an approximately constant stiffness during its attachment period to the microtubule. This stiffness increases with the distance between the microtubule's axis and the kinesin's attachment point on the surface. This variability leads to heterogeneity in motor force production originating in the heterogeneity in attachment geometry. The force production profile is asymmetric and highly heterogeneous.
In our work, we studied gliding microtubules whose movement is only opposed by viscous drag forces. Velocity fluctuations in a viscous medium will lead to a loss of efficiency on the order of our heterogeneity coefficient. If loads increase, e.g. due to the presence of cargo, the heterogeneity in force production will prevent homogeneous distribution of load among motors, and thereby prevent uniform loading with optimal force. This situation is well understood for cargo transport in vivo, where a small number of kinesins collectively pull cargo along microtubules.
An implication of the above considerations is that obtaining a more uniform attachment geometry via a method to position the motors directly beneath the microtubule would aid the propulsion of the microtubule. Indeed, muscle, one of the most efficient arrays of molecular motors, features precise alignment of these motors through the arrangement of thick and thin filaments.
These lessons are critical for the design of future nanoactuators and molecular motor-based devices. Although individual components such as kinesin motors may be able to operate with high energy efficiency, the efficiency of arrays and systems may suffer if these components are not appropriately integrated.
The results are described in detail in a manuscript submitted to Langmuir on 10/30/15 entitled: "Velocity Fluctuations in Kinesin-1 Gliding Motility Assays Originate in Motor Attachment Geometry Variations" by Henri Palacci,♯ Ofer Idan,♯ Megan J. Armstrong, Ashutosh Agarwal, Takahiro Nitta, and Henry Hess*.
2

Technology Transfer

ARO STIR Program

Final Report for W911NF-12-1-0384

Measurement of velocity fluctuations to infer attachment geometry and interactions of mechanically coupled molecular motors in one-dimensional arrays

PI: Dr. Henry Hess, Department of Biomedical Engineering, Columbia University, New York, NY, hh2374@columbia.edu

Period of Performance: 20 August 2012 – 19 May 2013

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