Novel Method for Screening the Electric Charge of Bio-Molecular Motors in Vitro

Ramiz Alejilat
Supervisor: Dr. Mitra Shojania-Feizabadi
Physics Department, Seton Hall University

Abstract

A better understanding of the nature of the electrostatic interactions between molecular motors and microtubules requires a thorough examination of, not only the charge of microtubules, but molecular motors as well. Several reported studies have been conducted to screen and evaluate the charge of microtubules *in vitro*. However, our knowledge about the electric charge of molecular motors is still limited to the evaluation of the association rate they express in conjunction with microtubules. With the goal of screening and evaluating the charge that molecular motors carry, we have designed a nanotechnology-based method. In this approach, the charge of motors can be screened *in vitro* by monitoring their behavior inside a uniform electric field. This novel *in vitro* approach minimizes the cellular factors that can potentially interfere with evaluating the charge of motors, leading to a better capability of evaluating the charge of these bio-motors in a more exclusive framework.

Introduction and Background

Biomolecular motors are one of the cellular components carrying a significant role in cell functions. Single molecule biophysical studies have made key contributions in understanding the intracellular functions of motors. Extensive studies in this field have revealed the diverse roles motors carry, ranging from cellular transportation to mitosis and cell division. Kinesin motors work in association with microtubules using them as their tracks to carry out cellular tasks. Specifically, Kinesin-1 are known for their ability to move along microtubules, mainly contributing to cellular transportation. New evidence indicates that the functions of motors can be regulated by structural specifications of microtubules. The changes in these specifications strengthen the possibility that the function of motors can be cell specific. Motor-microtubule interactions are poorly understood. Some findings propose electrostatic interactions between motors and microtubules may have a critical role in regulating the motors' functions. Most reported studies focus on the different electric charges of tubulin, and less attention has been given to better understand the charges that molecular motors carry.

Analytical Design and Method

In a set of parallel studies, we observed and analyzed the behavior of Kinesin-1 in a uniform electric field.

- 1 μm microsphere beads, 0.5 μm in diameter and density of 2 gram/cm3, were combined in an aqueous solution. Due to the high concentration, the beads were diluted 10^4 times.
- The concentrations used were 0.25 mg/mL and 0.5 mg/mL.
- The mixture was either immediately used or incubated for 30 minutes at room temperature, creating an environment for the motors to attach to the beads.
- 1-1.2 μL of the un-incubated beads or motor-covered beads were transferred to the micro-capacitor slide.
- The slide was covered with a clean cover slip and sealed on all edges.
- To create a uniform electric field, a microscope slide-based capacitor was used.
- The slide was coated in Indium-Tin Oxide. The uncoated gap in the center was 150 μm.
- A DC power supply was connected, creating a uniform electric field. The voltage was 1.5 V, producing an electric field of magnitude 100 V/cm.
- A dark-field Nikon microscope with an oil immersion objective lens was used to observe the beads.
- The microscope was connected to a camera where the movement of individual beads were recorded.
- The videos taken were analyzed using the program ImageJ.
- The displacement of the different beads after certain time frames were measured and charted.

Figure 1

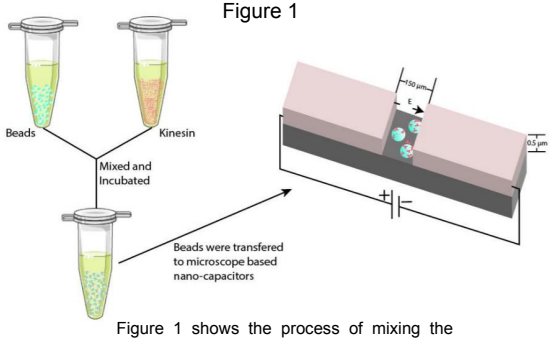
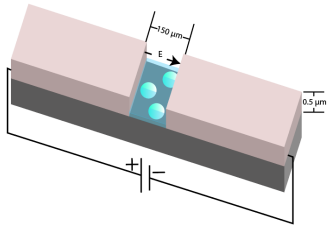
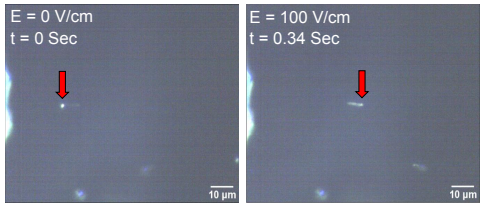


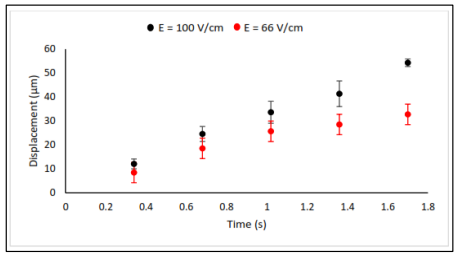
Figure 1 shows the process of mixing the beads with kinesin and applying it to the micro-capacitor slide.

Results and Discussion





A



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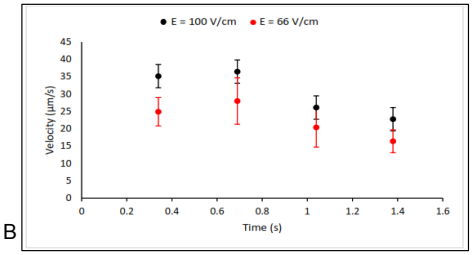

First row: Left : Represents the schematic view of the microscope slide nano-capacitor and the beads between electrodes. Right: Represents the movement of a bead in the absence of kinesin protein as the electric field is implemented. The figure shows a bead moving from the left of the screen to the right once an electric field of 100V/cm was applied over a period of .34 seconds.

Second row: In a set of control experiments, these graphs show the displacement (A) and the velocity (B) of beads in a kinesin-free environment and under the electric fields of 100 V/cm (black) and 66 V/cm (red). While the electric fields are calculated in the absence of the media, the actual value of the electric fields are slightly less due to the presence of media.

We also monitored the displacement of uncoated kinesin beads, when the kinesin is only in the media, and coated beads, when the beads were incubated with the kinesin protein. Our current data shows that the displacement of beads changes as we add kinesin protein. The displacement of kinesin coated beads are significantly less than that of uncoated beads. This is evidence that the negative charge of beads is reduced and as a result, they travel a shorter distance while under the influence of the electric field.

Conclusion

While among other factors, electrostatic interactions enables the kinesin-tubulin association, but the knowledge about this interaction in vitro is limited. This new approach was based on monitoring the behavior of kinesin-coated microsphere beads under the influence of a uniform electric field. The observational results confirm that this approach can be considered a method to detect and evaluate the charge of molecular motors. This experimental design, which relies on our capabilities in the area of nano-technology, provides us with a better insight about biophysical properties of biomolecular motors.

References and Acknowledgements

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We acknowledge funding **from New Jersey Space Grant Consortium for Ramiz Alejilat**.