The Effect of Mutated K253N on Kinesin's Speed

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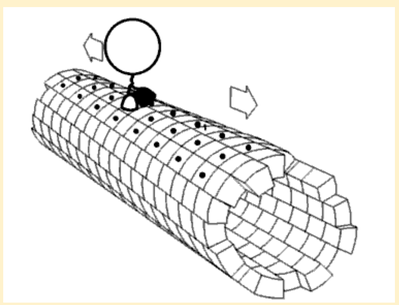
Abstract

Motor proteins, like kinesins, dyneins, and myosins, are enzymes that convert chemical energy into motion to transport large cellular components that cannot diffuse by themselves. Both kinesins and dyneins provide scaffolding for a large number of motor protein functions such as cell trafficking, cell division, and muscle contraction[1]. Because of their important roles, the malfunctioning of kinesins is associated with a variety of different diseases. Hereditary Spastic Paraplegia (HSP) is one of the neurodegenerative diseases associated with mutated conventional kinesin genes. Here, we investigate mutation K253N, one of the mutations of kinesin-1 gene associated with HSP. This work aims to uncover the molecular basis for the negative effect of mutation K253N on kinesin's speed.

Theoretical Background

- The conventional kinesin-1 is encoded by KIF5A gene. Since kinesin-1 is the major motor protein in neurofilament precursors, disruption of cargo delivery due to malfunctioning of kinesin-1 to the axon tip is likely the cause of neurodegeneration[3]
  - Kinesin superfamily protein (KIFs) defects can lead to several diseases such as peripheral neuropathy and Alzheimer's disease, in addition to HSP[2]
  - HSP is caused by malfunction in the domain inheritance of locus SPG10. This is caused by malfunction of two mechanisms that reduce gross cargo flux and lead to insufficient synapse supply[3]
  - For these reasons, the decrease in speed and processing of kinesin K253N is associated with HSP. Researching kinesin further will give us insight into why this disease develops

Structure and Function of Kinesin



This image shows the molecular transport of cargoes by motor proteins along a microtubule. These proteins include myosins, kinesins, and dyneins. This research investigates how the kinesin K253N converts chemical energy into motion. Image courtesy of Kolomeisky Group, Rice University

- Kinesins have 2 identical parts that each have an ATP and MT-binding site, and move in a coordinated, rotary motion
  - Kinesin transports organelles well because it has a flexible organelle-binding domain and doesn't hydrolyze ATP unless it is bound

GROMACS for MD Simulations

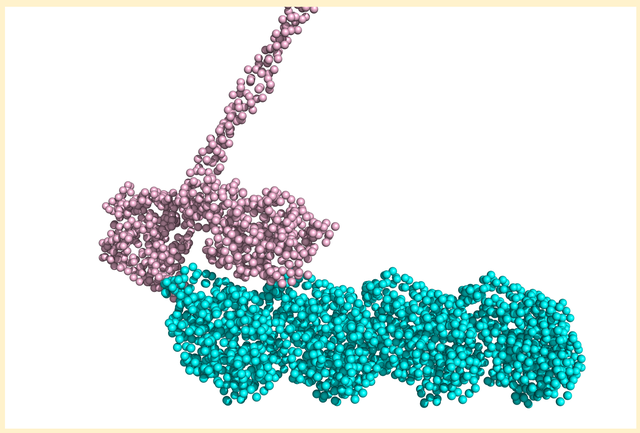
- Completion of three tutorials of GROMACS (Groningen Machine for Chemical Simulations) was necessary for this experiment. The tutorials on lysozyme in water, umbrella sampling, and free energy calculations were completed. The steps to producing a molecular dynamics (MD) simulation in GROMACS are as follows:
- **1) Generate topology.** The process starts with downloading a PDB (protein data bank) file of the selected protein. Files describing the protein's topology, atom coordinates, and position restraints (if any) are then generated and a force field is chosen.
- **2) Define box, solvate, and add ions.** Dimensions of the box holding the protein are set. In order to avoid periodicity and reference issues, pulling a distance less than half of the box length is necessary in umbrella sampling. Also in this step, a solvent and counter ions are added to charge balance the system (if necessary).
- **3) Energy minimization and equilibration.** Structures must be relaxed through energy minimization to avoid steric clashes. The structures are then equilibrated under constant pressure and/or volume, called NPT and NVT equilibration, respectively. Steepest descents energy minimization is frequently used in this step.
- **4) Production MD.** This data collection step includes the processing of the topology, coordinates, and molecular dynamics parameters to produce the desired data. It is oftentimes run in parallel on a cluster to speed up the process.
- **5) Data Analysis.** Many different techniques are used to analyze data in GROMACS. Some techniques include taking the root-mean-square deviation (RMSD) to compare two structures, taking the radius of gyration (R_g) along a reaction coordinate, and extracting thermodynamic quantities from potential of mean force (PMF) curves

Materials/Methods

- In our model, nonbonded interactions of mutation K253N were set to zero for 20 trajectories and were doubled for another 20 trajectories
  - Our model was developed using the SMOG (Structure-based models for biomolecules) website[6] from Rice and Northeastern University
  - We used a C-alpha coarse-grained model of Kinesin developed in SMOG
- We ran our model using a special version of GROMACS 4.5.4
  - We took 1000 data points of 50 nanoseconds each for 50 million total steps
  - 20 trajectories with K253N interactions turned off and 20 with K253N interactions doubled were simulated and compared to wild-type simulations
  - Umbrella sampling was used, and the temperature was set to 300K
- Energy difference (ΔE) of the ATP binding pocket is found by analyzing the gyration radius (R_g) of all trajectories
  - An R_g of above and below 1 indicates an open and closed ATP binding conformation, respectively
  - ΔE can be extrapolated from a negative natural logarithm probability distribution of R_g

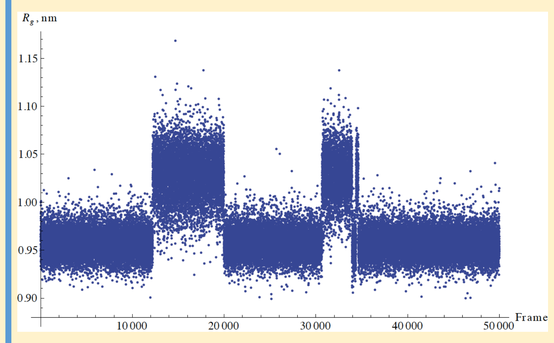
Coarse-Grained Model of Kinesin-1

- Coarse-graining is the practice of using simplified descriptions through the integration of many degrees of freedom into only a few[4]
  - Elastic network models (ENMs) represent the system through a network of beads[4]
  - In the K253N model, we represent each amino acid as a single bead in the C-alpha position
  - Coarse-grained, C-alpha models have been used in the past to determine topological frustration in proteins[5]
- There are different types of coarse-grained models. Kinetic and stochastic models predict mean velocity as a function of load force and ATP (adenosine-triphosphate) concentration
  - These parameters are measured to see the correlation between ATP binding conformation and kinesin's speed
  - Optical trap spectrometry is a successful method in which the motor drags a molecular bead out of a trap with a series of discrete steps. The effect of load forces on step length and velocity can be found using this method

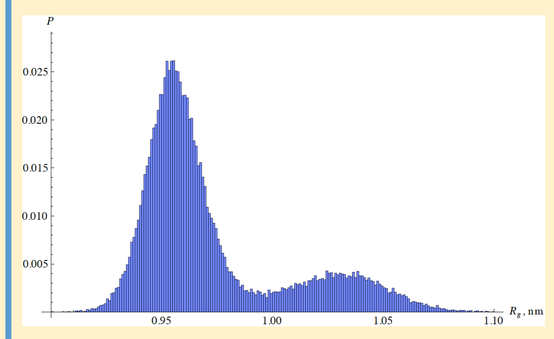


This image shows our coarse-grained model of kinesin-1 (pink) walking along a microtubule (turquoise). Kinesin-1 moves with a hand-over-hand motion, binding and unbinding to the microtubule each step

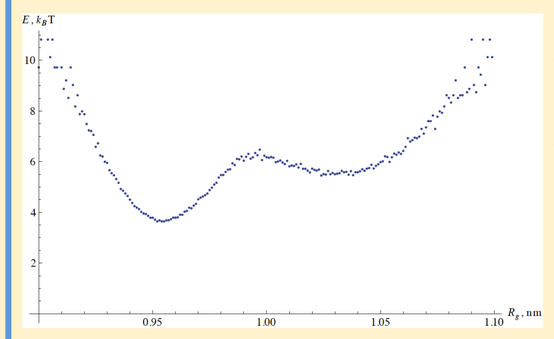
Results and Conclusion



This image shows an R_g plot along the reaction coordinate for a wild-type kinesin-1. The data clusters above and below 1 nm indicate a transition between open and closed ATP binding conformations



This figure, which is for the same wild-type kinesin-1 as above, is a probability distribution histogram of R_g. It shows that kinesin-1 is more likely to be in a closed ATP binding conformation



This plot shows the energy as a function of gyration radius. It was obtained by taking the inverse logarithm of the probability distribution. The energy difference (ΔE) is taken by subtracting the two local minima

- Although the results are still being analyzed, data averaged from 30 wild-type kinesin-1 trajectories gave a ΔE of 1.865 k_BT
  - The next part of the study will be to generate plots and extrapolate ΔE for each of the 40 mutant trajectories; 20 trajectories for zero nonbonded interactions in mutation K253N and 20 for doubled nonbonded interactions
- I learned many skills this summer, including how to use GROMACS and compiling programs in supercomputers
  - In addition to learning GROMACS language and commands, I gained valuable knowledge about the process of running chemical simulations
  - Compiling GROMACS in the supercomputer Davinci gave me experience with Davinci language, as well as basic Linux commands
  - After learning how to create models of kinesin mutants, I was able to study the energy difference between conformations of the ATP binding pocket

References and Acknowledgement

I would like to thank the National Science Foundation (grant number PHY-1427654), the CTBP at Rice University, and University of Houston for funding the Frontiers in Science (FIS) summer program. Special thanks to Mikita Misiura for being a great mentor, Dr. Kolomeisky for leading the research, Dr. Cheung for running the FIS program, and the other FIS interns for helpful discussions.

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