

ABSTRACT

Intracellular transportation involves multiple molecular proteins working together in coordination. These proteins are of different types, i.e. they have different chemical as well as physical properties. Understanding transport properties of these molecules individually as well as of their ensembles help understand the underlying mechanism of genesis of neurodegenerative diseases.

Various studies have been carried out to characterize motion of a molecular cargo *in-vivo* as well as *in-vitro*. Analytical approaches and Monte-Carlo Simulations used to study how motor-proteins coordinate the transportation of a cargo are either too simplistic in nature or require high computational power, at the same time do not capture the rare event scenarios. To overcome these challenges, semi-analytical approaches have proven to be quite effective for studying biologically relevant quantities while enabling us to observe the occurrence of rare events.

So far, semi-analytical approaches have been developed for studying the transportation of a shared cargo with ensemble of motor protein of only one species type. In these approaches, the movement of cargo is restricted to only one direction on microtubule lattice. Further work has been done to extend this approach to the case when some of the motors are mutated while still being restricted to one directional motion of cargo. These approaches, while being less computationally intensive compared to Monte-Carlo simulations, produce comparable results to any other techniques available for simulating single species, unidirectional transportation. They also give insights about some of the rare events occurring which other techniques fail to capture. This provides the motivation to develop similar approach and extend the existing semi-analytical framework for bi-direction intracellular transportation.

We develop a more generalized approach which enables us to study the transportation of cargo by an ensemble of two species motor molecules. This enables us to study biologically relevant quantities such as average run-length and average velocity of the cargo molecule while taking into account bi-directional motion of ensemble. Added direction of motion and forces gives rise to the change in behavior of ensemble and makes the extension of the single species, unidirectional case to a two species, bi-directional case a non-trivial task.

We begin by defining the transition probabilities of change in absolute configurations of ensemble. Since the number of absolute configurations of an ensemble, and hence their combinations, are infinite, this makes the problem intractable. We determine an equivalence class of various absolute configuration by using the relative configurations defined by the relative distances of the motors. We prove that the number of such relative configurations are finite. We further prove that several biologically relevant quantities can be calculated using the relative configurations. The technique has been instantiated by calculating these quantities for an ensemble of motors consisting of Kinesin and Dynein and some of the key results are presented.

COMPUTATIONS^[1,2] AND PARAMETERS USED³

PARAMETERS	UNITS	KINESIN	DYNEIN
Spring Constant	pN/nm	0.32	0.32
Step Size	nm	8	8
Stall Force	pN	5	1.25
Rest Stalk Length	nm	110	50
Rate of attachment	per motor per second	5	5
Unloaded Run Length	nm		689

$$\begin{aligned} \text{Type 1 species} &\rightarrow (\dots 0 \ 0 \ 1 \ 1 \ 0 \ 1 \ \dots) \\ \text{Type 2 species} &\rightarrow (\dots 1 \ 0 \ 1 \ 0 \ 0 \ 0 \ \dots) \end{aligned} \quad \rightarrow \quad \begin{pmatrix} 0 & 0 & 0 & 1 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \end{pmatrix}$$

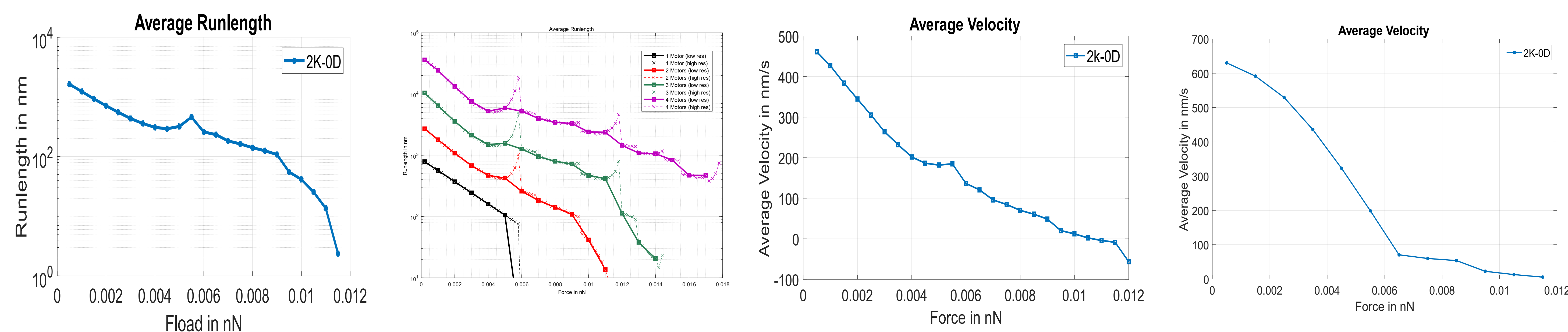
Infinite dimensional representation of configuration

Converted to a finite dimensional Markov model by taking projections and defining equivalence class of relative configurations

$$\frac{\partial}{\partial t} P_{rel}(\sigma, t) = -P_{rel}(\sigma, t) \sum_{\sigma' \in Q} \lambda_{rel}(\sigma', \sigma) + \sum_{\sigma' \in Q} \lambda_{rel}(\sigma, \sigma') P_{rel}(\sigma', t) \Rightarrow \frac{d}{dt} P(t) = AP(t) \Rightarrow P(t) = e^{A(t-t_0)} P(t_0)$$

- Projection possible because of finite number of relative configurations which is a consequence of finite extent
- Probabilities of each relative configurations are found using the above master equation
- $\lambda_{rel}(\sigma', \sigma)$ are the transition rates from one relative configuration to other. Rates are computed using stepping, attachment and detachment rates of both type of species
- Type 1 species can step only in forward direction and type 2 species can only step in backward direction
- Both species have different load force dependent stepping and detachment rates

VALIDATION



With Bi-Directional Model

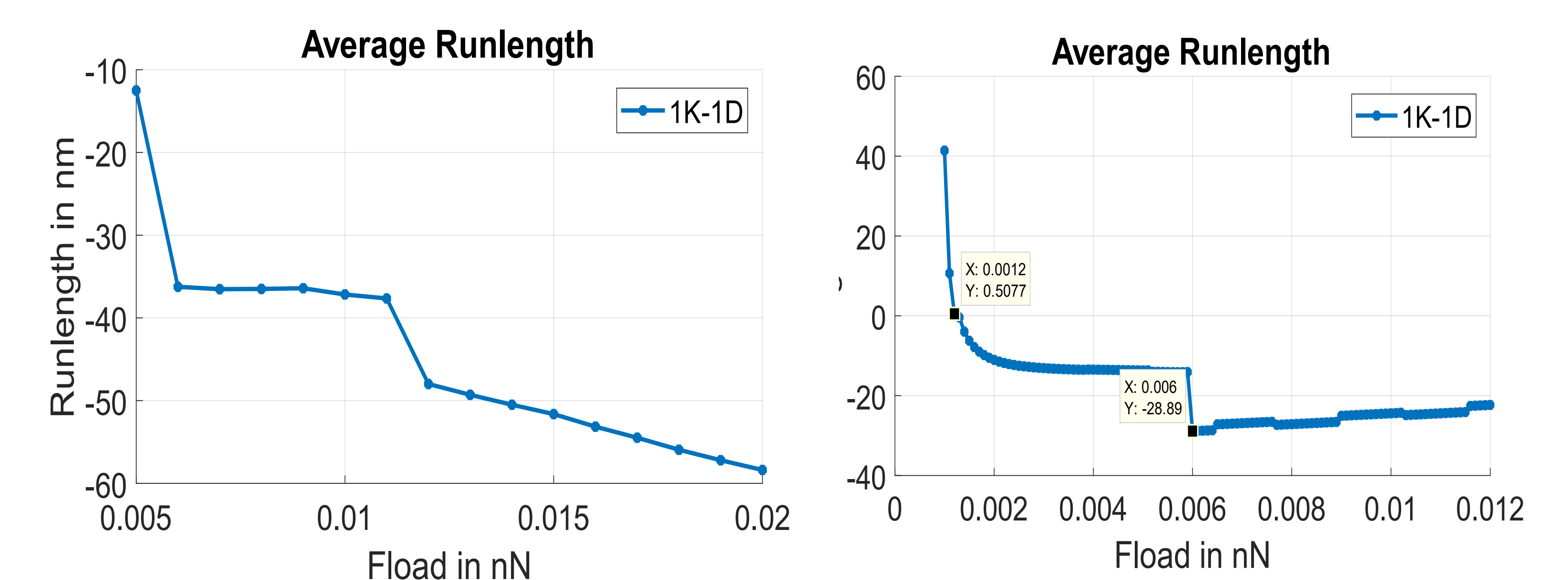
With Unidirectional Model¹

With Bi-Directional Model

With Unidirectional Model¹

- For validation of the bi-directional model, the number of type 2 species were taken to be 0
- Model Validated against previous Unidirectional Model using Kinesin Parameters
- Bi-directional model gives similar trends in Average run length and Average Velocity as compared to unidirectional model

SIMULATION RESULTS



Ensemble of two motor molecules with similar parameters but opposite directionality

Ensemble of Kinesin and Dynein Using Realistic Parameters (very high resolution)

- Typical values of run length at very high load forces (0.1 nN) were found to be 250 nm for 1 Kinesin 1 Dynein Ensemble as compared to 690 nm in case of 2 Kinesin Ensemble

CONCLUSIONS

- This paper presents a generalized framework for evaluation of properties of bi-directional intracellular transport
- Possible to Incorporate 2 different type of species with bi-directional motion capability
- Preliminary results indicate that load force decides the direction, direction switching observed when load force is equal to minimum of the two stalling forces
- Real nature of bi-directional ensemble explored

FUTURE DIRECTIONS

- More realistic Dynein models with varied step length to be incorporated
- Both forward and backward moving probabilities of Dynein to be incorporated
- Comparison with Monte Carlo simulations and experimental results
- Simulating models which represent *in-vivo* conditions with changes in Temperature, ATP Concentration etc. and with an option of reversal of direction of force as soon as direction of movement changes

REFERENCES

- [1] Materassi D, Roychowdhury S, Hays T, and Salapaka M: An exact approach for studying cargo transport by an ensemble of molecular motors. *BMC biophysics* (2013)
- [2] Bhabhan S, Materassi D, Li M, Hays T, and Salapaka M: Interrogating Emergent Transport Properties for Molecular Motor Ensembles: A Semianalytical Approach. *PLoS Computational Biology* (2016)
- [3] Hong W, Takshak A., Osunbayo O, Kunwar A, Vershinin M: The Effect of Temperature on Microtubule-Based Transport by Cytoplasmic Dynein and Kinesin-1 Motors. *Biophysical Journal* (2015)