Modeling Neck Linker Extension in Kinesin.

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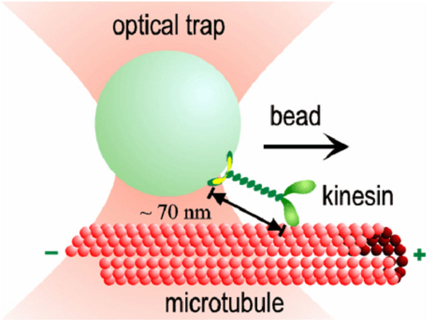
Joint work with Matthew Kutys, John Hughes, and William Hancock.

Overview.

- The Kinesin Molecular Motor.
- Important Quantities–Asymptotic Velocity, Effective Diffusion, Processivity.
- Common Models.
- Our Model.
- A Numerical Strategy.
- Extensions and Current Work.

An Artist's Rendering.

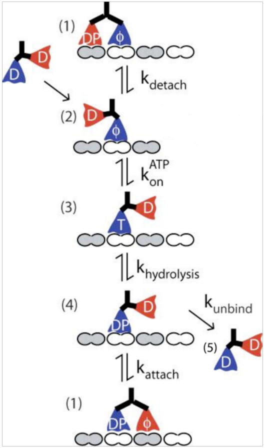


Block Lab:http://www.stanford.edu/group/blocklab/kinesin/kinesin.html

The Important Biological Points.

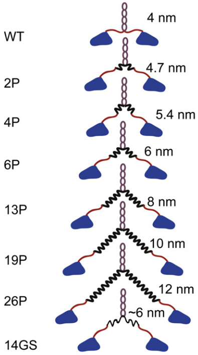
- "Hand over hand" stepping mechanism.
- 8 nanometer steps with 1 ATP per step.
- Length of step determined by the physical structure of microtubule.
- Back steps are rare.
- Kinetics + Constrained Diffusion.
  - Free head detachment.
  - ATP binding.
  - ATP hydrolysis.
  - Free head attachment.

The Kinesin Cartoon.

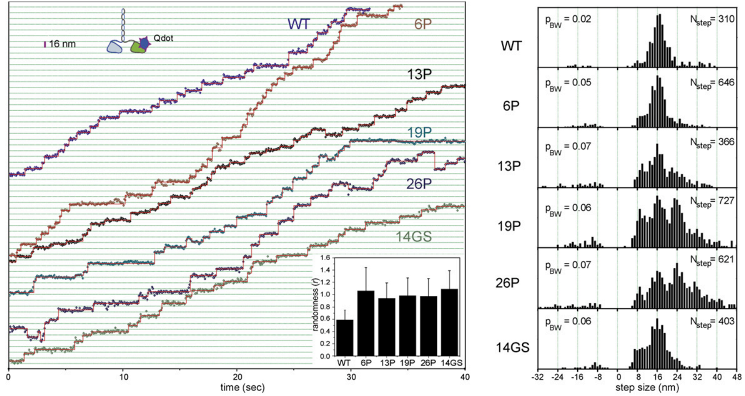


Engineered Motors.

- Extensions can range from less than 1 nm up to 12 nm.
- Hackney and Hancock–extensions reduced processivity.
- Hancock–velocity was reduced.
- Yildiz et al–processivity was unaffected and velocity was reduced.



Necklinker Extension.



Yildiz, A. and Tomishige, M. and Gennerich, A. and Vale, R.D.

Intramolecular Strain Coordinates Kinesin Stepping Behavior along Microtubules.

Kinesin Measurement.

- Different measurement methods.
  - Laser Trap.
  - FRET.
  - FIONA.
- What data is collected?
  - Position.
  - Time of Run.
  - Size of Steps.

Important Quantities of Interest.

- **Asymptotic Velocity**

$$V_a = \lim_{t\to\infty} \frac{E[X(t)]}{t} \quad or \quad V_a = \lim_{t\to\infty} \frac{X(t)}{t}$$

- **Effective Diffusion**

$$D_{eff} = \lim_{t\to\infty} \frac{Var[X(t)]}{2t}$$

  or the quantity which ensures

$$\frac{X(t) - V_a t}{\sqrt{2D_{eff}\, t}}$$

  converges to a standard normal.

- **Randomness Parameter**

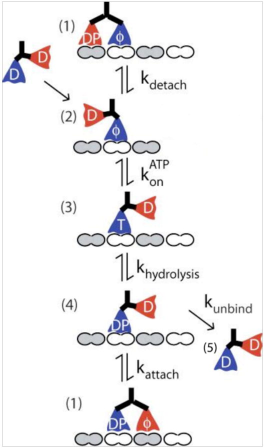
$$R = \frac{2D_{eff}}{LV_a}$$

- **Processivity**
  ν the number of random steps taken before detachment.

The Models.

Pure kinetics model–a discrete space Markov chain.

- Fails to account for the physical movement of heads.



The Models.

Stochastic Differential Equation Model

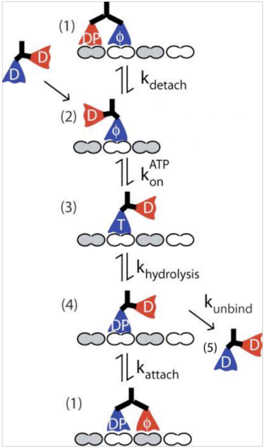
- Brownian particle in a periodic potential.
- $dX(t) = a(X(t))dt + \sigma dB(t)$
- Fails to account for two individual heads.
- Fails to coordinate physical movement and chemical kinetics.

The Models.

Flashing Ratchet

- $dX(t) = a_{K(t)}(X(t))dt + \sigma dB(t)$
- Accounts for both chemical and physical states.
- How can these be coordinated?

The Kinesin Cartoon.



Our Model.

- What about incorporating diffusion of the freehead into the model?
- State 1 corresponds to having both heads bound.
- State 2 corresponds to the head having become free
  Tethered diffusion with a negative or neutral bias.
- State 3 and state 4 mean ATP has been bound
  A conformational change causes there to be a forward bias and less compliant spring.

Our Model.

- The position of the free motor head is governed by the following equation.

$$Y(t) = y + \int_0^t a_{K(s)}(Y(s))ds + \sigma B(s)$$

  where $K(t)$ is the process corresponding to state events.

- Associate with each binding site a binding process

$$N_j \left(\int_0^t g_j \left(Y(s) \right) ds \right)$$

  where the N_j are independent standard Poisson processes (independent of B also).

- The time until we return to (chemical) state one (τ) would then be the time for one of these clocks to fire.

- We define $Y(\tau)$ to be the location of the binding site associated with the binding process which fires first.

Renewal-Reward Processes.

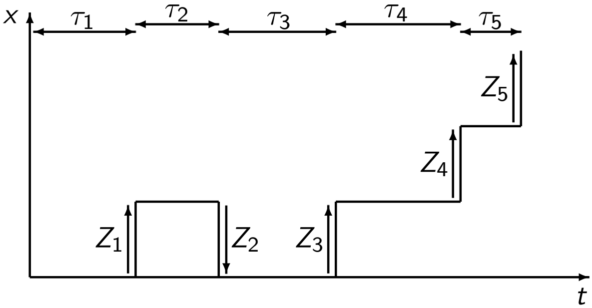
- $Z_i, \;\; i = 1, 2, \ldots$ with mean μ_z and variance σ_z^2.

$$X(t) = \sum_{i=1}^{N(t)} Z_i$$

where $N(t)$ is a renewal process.

  - $N(t) = \max\{n : \sum_{i=1}^{n} \tau_i \leq t\}$
  - Time between events are independent and identically distributed, $\tau_i, \;\; i = 1, 2, \ldots.$ $(\tau_0 = 0)$.
  - The τ_i have finite mean (μ_τ) and variance (σ_τ^2).

Renewal-Reward Process.



Limits for Renewal-Reward Process.

For motor with backwards/forward steps,

- $$V_a = \lim_{t\to\infty} \frac{LX(t)}{t} = \frac{L\mu_z}{\mu_T}$$

- $$D_{eff} = \lim_{t\to\infty} \frac{L^2 Var[X(t)]}{2t} = \frac{L^2}{2}\left(\frac{\sigma_Z^2}{\mu_T} + \frac{\mu_z^2\sigma_T^2}{\mu_T^3}\right)$$

Functional Central Limit Theorem.

Define

$$S(t) = \sum_{i=0}^{\lfloor t \rfloor} Z_i \quad T(t) = \sum_{i=0}^{\lfloor t \rfloor} \tau_i$$

$$n^{-1/2} \begin{pmatrix} S(nt) - \mu_Z nt \\ T(nt) - \mu_\tau nt \end{pmatrix} \Rightarrow \begin{pmatrix} B_1(t) \\ B_2(t) \end{pmatrix}$$

where

$$\Sigma = \begin{pmatrix} \sigma_Z^2 & 0 \\ 0 & \sigma_\tau^2 \end{pmatrix}$$

Now, if we define

$$X_n(t) = n^{-1/2} \left(S(T^{-1}(nt)) - \frac{\mu_Z}{\mu_\tau} nt \right)$$

and we apply Theorem 13.7.3 from Whitt; we obtain

$$X_n(t) \Rightarrow B_1\left(\frac{t}{\mu_\tau}\right) - \frac{\mu_Z}{\mu_\tau} B_2\left(\frac{t}{\mu_\tau}\right).$$

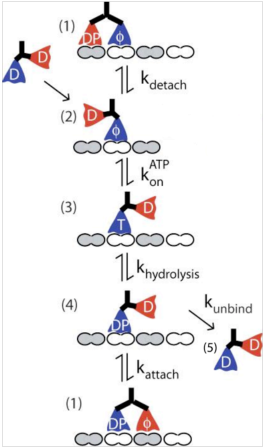
FCLT for Renewal-Reward.

- Note that $X(t) = S(T^{-1}(t))$
- This is equivalent in law to

$$X_n(t) = n^{-1/2}\left(X(nt) - \frac{\mu_z}{\mu_\tau} nt\right) \Rightarrow \sqrt{\frac{\sigma_Z^2}{\mu_\tau} + \frac{\mu_z^2\sigma_\tau^2}{\mu_\tau^3}}B(t)$$

- $$X(nt) \approx \frac{\mu_z}{\mu_\tau} nt + n^{1/2}\sqrt{\frac{\sigma_Z^2}{\mu_\tau} + \frac{\mu_z^2\sigma_\tau^2}{\mu_\tau^3}}B(t)$$

The Kinesin Cartoon.



Kinetic Model.

Relabel the states. Negative means front head became detached first.

$$Q = \left(\begin{array}{c|c} A & B \\ \hline 0 & 0 \end{array} \right)$$

$$\mathbf{A} = \begin{pmatrix} k_{1_+,1_+} & k_{1_+,2_+} & 0 & 0 & k_{1_+,4_-} & 0 & 0 \\ 0 & k_{2_+,2_+} & k_{2_+,3_+} & 0 & 0 & 0 & 0 \\ 0 & k_{3_+,2_+} & k_{3_+,3_+} & k_{3_+,4_+} & 0 & 0 & 0 \\ 0 & 0 & k_{4_+,3_+} & k_{4_+,4_+} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_{4_-,4_-} & k_{4_-,3_-} & 0 \\ 0 & 0 & 0 & 0 & k_{3_-,4_-} & k_{3_-,3_-} & k_{3_-,2_-} \\ 0 & 0 & 0 & 0 & 0 & k_{2_-,3_-} & k_{2_-,2_-} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{1}$$

and

$$\mathbf{B} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & K_{2_+,1_*} & 0 \\ 0 & 0 & 0 \\ k_{4_+,1_{++}} & 0 & 0 \\ 0 & k_{4_-,1_*} & 0 \\ 0 & 0 & 0 \\ 0 & 0 & k_{2_-,1_-} \end{pmatrix}. \tag{2}$$

Aggregated States of Markov Chains.

- Wang and Qian on kinetic models for motors.
- Milescu et al on MLE for motor dwell time.
- Fredkin and Rice a comprehensive look.
- Colquhoun and Hawkes with ion channels.
- Queueing Literataure–Asmussen, Neuts+others

Formulas.

Using this formulation, the transition matrix can, under suitable conditions, be written as

$$P(t) = e^{Qt}$$

In the decomposition into submatrices, this can be simplified into

$$e^{Qt} = \begin{pmatrix} e^{At} & e^{At}A^{-1}B - A^{-1}B \\ 0 & I \end{pmatrix}$$

Formulas.

This also allows for the time until absorption (τ_i)

$$\begin{aligned} F(t) &= P(X(t) = 1_- \text{ or } 1_{++}) \\ &= 1 - P(X(t) \neq 1_- \text{ or } 1_{++}) \\ &= 1 - ae^{At}\mathbf{1}' \end{aligned}$$

The mean and variance for τ_i are readily available.

$$\mu_\tau = -aA^{-1}\mathbf{1}'$$

and

$$\sigma_\tau^2 = 2aA^{-1}A^{-1}\mathbf{1}' - \left(\nu A^{-1}\mathbf{1}'\right)^2$$

Formulas.

- We can also find the probability of a forward or backward step.
-

$$\begin{aligned} P(X(\tau) = 1_{++}, \tau \le t) &= P(Z = 1, \tau \le t) \\ &= a\left(I - e^{At}\right)\left(-A^{-1}B\right)c' \end{aligned}$$

-

$$P(Z = 1) = \lim_{t \to \infty} P(Z = 1, \tau \le t) = a\left(-A^{-1}B\right)c'$$

- This allows us to calculate μ_z and σ_z^2.

Formulas.

- The covariance can be calculated from the joint distribution.
- $$P(Z=z,\tau \le t) = a\left(I - e^{At}\right)\left(-A^{-1}B\right)c_z'$$
- From this we obtain the density
$$f(z,t) = ae^{At}Bc_z'$$
- This allows us to calculate the covariance, $\sigma_{Z,\tau}$.
$$-aA^{-1}Bd'$$
- Note we can also calculate the conditional density
$$f(z|t) = \frac{f(z,t)}{f(t)} = \frac{ae^{At}Bc_z'}{-aAe^{At}\mathbf{1}'}$$

Dependence.

- Thus, $X_n(t) \Rightarrow B_1\left(\frac{t}{\mu_\tau}\right) - \frac{\mu_Z}{\mu_\tau} B_2\left(\frac{t}{\mu_\tau}\right)$.
- We can mutliply out the diffusion coefficient of the resulting Brownian motion

$$\frac{1}{\mu_\tau^2}\begin{pmatrix} 1 & -\frac{\mu_Z}{\mu_\tau} \end{pmatrix}\begin{pmatrix} \sigma_Z^2 & \sigma_{Z,\tau} \\ \sigma_{Z,\tau} & \sigma_\tau^2 \end{pmatrix}\begin{pmatrix} 1 \\ -\frac{\mu_Z}{\mu_\tau} \end{pmatrix}$$

- So, $X_n(t) = n^{-1/2}\left(X(nt) - \frac{\mu_z}{\mu_\tau} nt\right) \Rightarrow \sqrt{\frac{\sigma_Z^2}{\mu_\tau} + \frac{\mu_z^2 \sigma_\tau^2}{\mu_\tau^3} - 2\frac{\mu_Z \sigma_{Z,\tau}}{\mu_\tau^2}}\, B(t)$
- This is equivalent in law to $X(nt) \approx \frac{\mu_z}{\mu_\tau} nt + n^{1/2}\sqrt{\frac{\sigma_Z^2}{\mu_\tau} + \frac{\mu_z^2 \sigma_\tau^2}{\mu_\tau^3} - 2\frac{\mu_Z \sigma_{Z,\tau}}{\mu_\tau^2}}\, B(t)$

A Matrix Approximation for Our Model.

We can approximate the evolution of a cycle using a similar matrix setup to the purely kinetic. The matrices will have the following block form:

$$\mathbf{A} = \begin{pmatrix} k_{1_+,1_+} & k_{1_+,2_+} & 0 & 0 & k_{1_+,4_-} & 0 & 0 \\ 0 & k_{2_+,2_+} & k_{2_+,3_+} & 0 & 0 & 0 & 0 \\ 0 & k_{3_+,2_+} & k_{3_+,3_+} & k_{3_+,4_+} & 0 & 0 & 0 \\ 0 & 0 & k_{4_+,3_+} & k_{4_+,4_+} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_{4_-,4_-} & k_{4_-,3_-} & 0 \\ 0 & 0 & 0 & 0 & k_{3_-,4_-} & k_{3_-,3_-} & k_{3_-,2_-} \\ 0 & 0 & 0 & 0 & 0 & k_{2_-,3_-} & k_{2_-,2_-} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{3}$$

and

$$\mathbf{B} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & K_{2_+,1_*} & 0 \\ 0 & 0 & 0 \\ k_{4_+,1_{++}} & 0 & 0 \\ 0 & k_{4_-,1_*} & 0 \\ 0 & 0 & 0 \\ 0 & 0 & k_{2_-,1_-} \end{pmatrix}. \tag{4}$$

A Submatrix.

$k_{2_+,2_+} =$

$$
\begin{array}{c c}
 & \begin{array}{cccccc} y_1 & y_2 & \cdots & \cdots & \cdots & y_n \end{array} \\
\begin{array}{c} y_1 \\ y_2 \\ \cdots \\ \cdots \\ y_n \end{array} &
\begin{pmatrix}
-\sum_{n+1} & \mathbf{L}_{1,2} & 0 & 0 & \cdots & 0 \\
\mathbf{L}_{2,1} & -\sum_{n+2} & \mathbf{L}_{2,3} & 0 & \cdots & \cdots \\
0 & \cdots & \cdots & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \mathbf{L}_{n-2,n-1} & -\sum_{n+(n-1)} & \mathbf{L}_{n-1,n} \\
0 & \cdots & \cdots & 0 & \mathbf{L}_{n,n-1} & -\sum_{2n}
\end{pmatrix}
\end{array}
$$

Necklinker Models (Drifts).

- $Y(t) = x + \int_0^t a_{K(s)}(Y(s))ds + \sigma B(s)$
- **Linear Spring**

$$a_k(y) = -\kappa(y - c)$$

- **WLC**

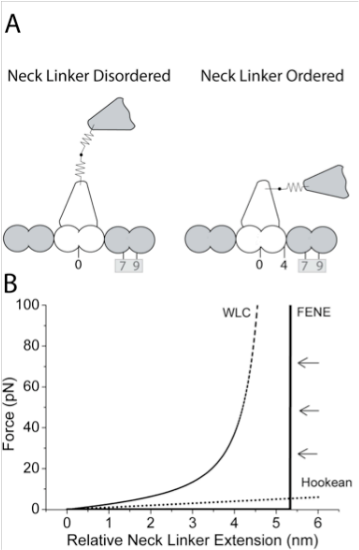
$$a_k(y) = \kappa \left(\frac{1}{4} \left(1 - \frac{y}{L_c} \right)^{-2} - \frac{1}{4} + \frac{y}{L_c} \right)$$

- **FENE**

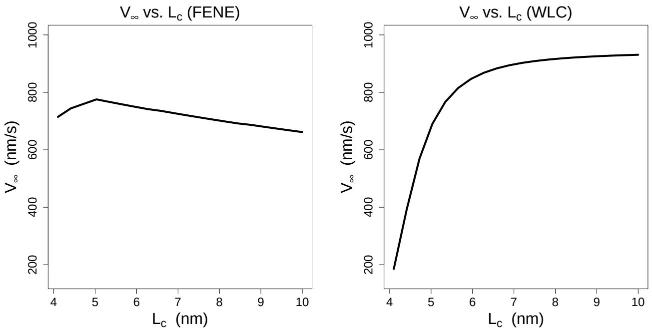
$$a_k(y) = -\kappa(y - c)$$

but with reflecting barriers at L_c and $-L_c$.

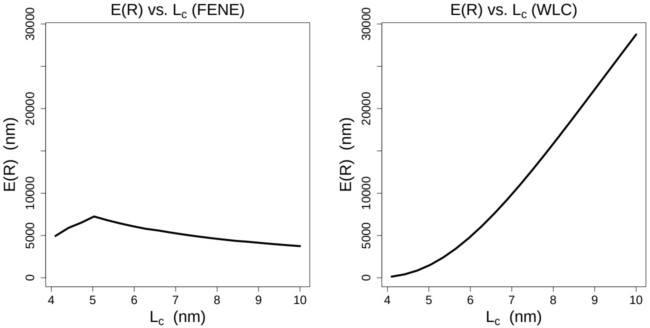
Necklinker Models (Drifts).



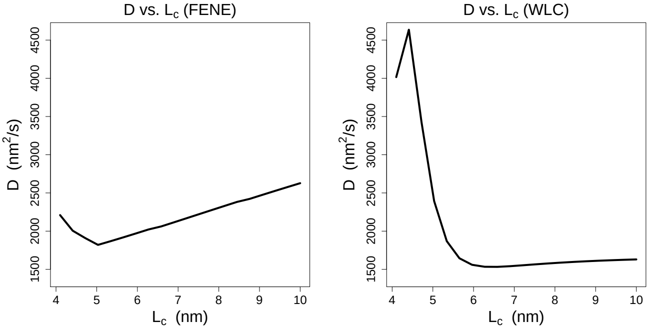
Velocity.


V_∞ vs. L_c (FENE)
V_∞ (nm/s)
200
400
600
800
1000
4
5
6
7
8
9
10
L_c (nm)
V_∞ vs. L_c (WLC)
V_∞ (nm/s)
200
400
600
800
1000
4
5
6
7
8
9
10
L_c (nm)

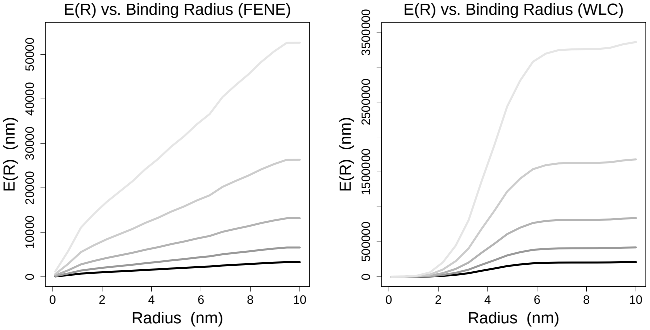
Run Length.


E(R) vs. Lc (FENE)
E(R) (nm)
0
5000
10000
20000
30000
4
5
6
7
8
9
10
Lc (nm)
E(R) vs. Lc (WLC)
E(R) (nm)
0
5000
10000
20000
30000
4
5
6
7
8
9
10
Lc (nm)

Effective Diffusion.



Binding Radius and Attachment Rate.

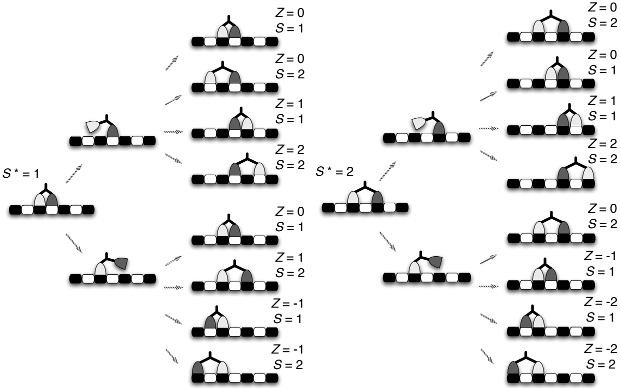


Summary for Different Spring Models.

- WLC.
    - When allowed to extend to approximately 4nm, binding constant must be very high.
    - As neck linker is extended, velocity AND processivity increase.
- FENE.
    - Binding constant is reasonable.
    - As neck linker is extended, velocity and processivity decrease as expected.
- Possible Resolutions.
    - Projection is the problem.
    - Weak binding.
    - Mis-specficiation of neck linker.

Multiple Step Model.

- Heads are not necessarily one binding site away at the beginning of each cycle.
- Return to double binding changes initial conditions of next cycle.



Multiple Step Model.

The following forms a Markov chain

$$\begin{pmatrix} Z_i \\ \tau_i \\ S_i \end{pmatrix}$$

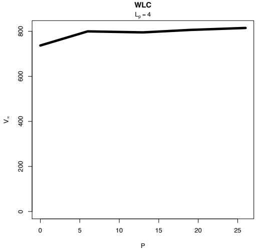
- S_i is a Markov chain describing the distance between heads after previous cycle.
- The position of the front head after a full cycle

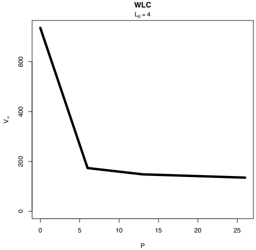
$$X(t) = \sum_{i=1}^{N(t)} Z_i$$

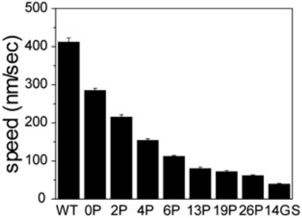
Multiple Step Model.

- Take advantage of the simplified structure; Z_i and τ_i depend on the last value of S.
- Calculate the stationary distribution of S_i using the matrix approximation.
- Can calculate the other moments based only on the conditional means and variances given S_{i-1}.
- Central Limit Theorem for stationary Markov chains will lead to FCLT for sums–the result is a bivariate Brownian motion
- We can still use Whitt to give us the correct FCLT.

Tension vs No Tension.







Future Directions.

- Modeling Laser Trap Experiments.
- How does this nanoscale model react to external (and fluctuating) forces?
- How about imposed forces?
- What happens when multiple motors are connected to the same cargo?

Acknowledgements.

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