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Supplemental Information

Diffusion as a Ruler: Modeling Kinesin Diffusion as a Length Sensor for Intraflagellar Transport

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TRANSITION MATRIX MODEL

Here we seek a more abstract model that can be analyzed mathematically to yield a more intuitive understanding of why the model works the way it does. To this end, we modeled the flagellum as a column vector N(t), with each element in the vector representing the number of motors at that location processing along the flagellum at time t. We then extended that vector to twice the length of the flagellum, with each element in the second half representing the number of motors diffusing at the corresponding location. Finally, we extended the vector by one element to represent the number of motors in the base. We can then represent the dynamics of the entire system using a stochastic matrix M such that M*N(t) = N(t+1).

Supplemental figure 1A shows an example transition matrix M representing the dynamics of a flagellum of length 4. To construct M, we need to consider several constraints. First, the number of motors in the system must be conserved, so the sum of the elements in the state vector N(t) must remain constant throughout all t. The columns can be thought of as the spread of a point source after one time step. Specifically, if the value of the state vector component at position j at time t is n_j , the transition matrix will redistribute those n_j motors into a new distribution, governed by the values in M. Since every motor needs to end up in some position (given conservation of total motor number), the entries in the whole column must sum to 1. The condition that each column in M must sum to 1 defines M as a left stochastic matrix. This property of the matrix will help us later determine the steady state of the system and solve the length control problem.

Second, the matrix must simulate active transport for the top half of the state vector, diffusion for the bottom half, and absorption/recruitment to send motors from the bottom value to the top value. Since we constructed the state vector such that the first L values represent bound (i.e. transporting) motors, the top left quadrant of the transition matrix M will represent the active transport dynamics. Active transport is simply moving some percent of motors one unit forward and keeping the remaining motors at their current position at each time step, so the active transport quadrant of the matrix will have positive values on the diagonal and one position under the diagonal.

The diffusion region of the transition matrix must apply to motors that have moved past position L in the state vector. This means that the lower right quadrant of the transition matrix M must simulate the dynamics of diffusion. We can incorporate the random walk nature of diffusion into this matrix by stating that the probability of staying in the same position is high, and the position of moving one position to either side is low. This simulates the Gaussian spread of a diffusing point source after a small time (we keep the time small so there is a negligible chance of diffusion two units away).

Notice that the first column incorporates the reflecting boundary condition that motors cannot go past the tip, so the odds of staying at the tip are the odds of not moving anywhere (here 0.98) plus the odds of moving past the tip and bouncing off (here 0.01). Also note that the way our state vector is constructed, motors diffusing in the direction of the base are going down the state vector towards lower rows. This matches the order in which vector elements representing diffusing kinesins are specific in the state vector

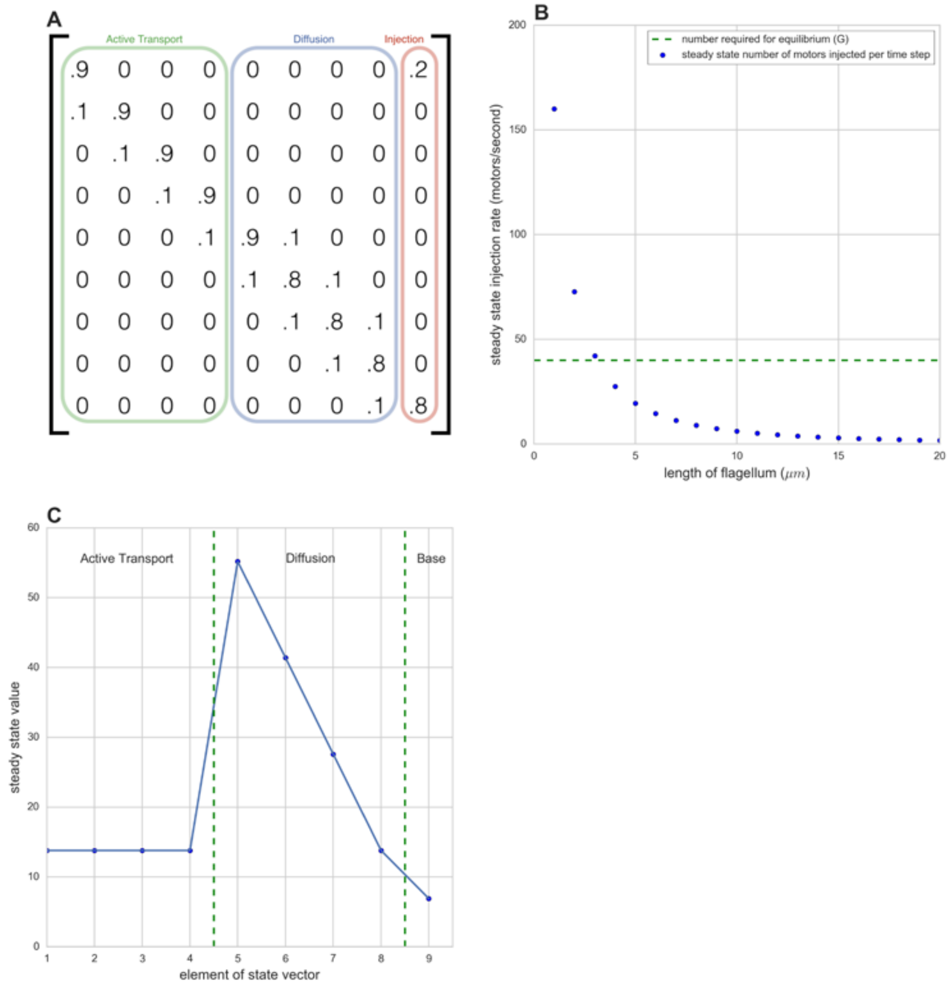
With the aforementioned elements of M specified, we are able to represent how the motors can actively transport to the tip, unbind, diffuse back to the base, and absorb at the base so that motors enter the inactive pool. We still need to add the final element of our dynamics into the matrix: injection. A simple way to do this is to assume that at each time step, the base sends p percent of the motors in the base back to the flagellum for active transport. This means that 1-p represents the proportion of motors that stay in the base. Such an assumption is a simplified representation of the quasi-periodic avalanching process, and may need to be relaxed in future simulations. The last column in M represents the spread of motors that were previously at the base. To incorporate avalanching and recruitment into this column, we simply make the column $[p\ 0\ 0\ \ldots\ 0\ 0\ 1\text{-}p]^T$, where p is the probability of a motor being injected.

Now all the columns in the matrix sum to 1, so the condition for being a stochastic matrix are satisfied. The probability of different states evolves in a strictly deterministic manner determined by successive matrix multiplications. For example, if the diffusion half of the state vector is $[0\ 1\ 0\ 0]^T$, applying M will result in a new state vector whose elements are real numbers in the range 0 to 1 that represent the probability of a motor occupying that position in the state vector. This makes sense physically in the assumption that there are a large number of motors in the system, and since the number is on the order of 200 motors, this is a reasonable approximation.

One limitation of this construction of the transition matrix is that it assumes a constant flagellum length. The length determines the size of the matrix, so to simulate length dynamics over time, we would need to continuously alter the size of the matrix. To avoid this inconvenience, we can instead directly calculate the steady state behavior as a function of flagellar length. The steady state solution N_{ss} must satisfy $M * N_{ss} = N_{ss}$, so N_{ss} is an eigenvector of M with eigenvalue 1. The Perron-Frobenius theorem states that the largest magnitude eigenvalue of stochastic, nonnegative, and irreducible matrix is always simple and equal to 1. Our motor transition matrix is stochastic (i.e. Markov) because the columns each sum to 1. It is nonnegative because all values are greater than or equal to zero. Finally, it is irreducible because each node has a path to get to every other node after some number of time steps. For example, a motor in the middle of active transport has a path leading through every subsequent active transport node, then it connects to a diffusion node, and each diffusion node is connected to a subsequent diffusion node, the last one connects to the base node, which connects to the first active transport node. This means we can apply the Perron-Frobenius theorem for nonnegative irreducible matrices to this stochastic matrix, proving that the eigenvalue of 1 always exists and is unique, and corresponds to a principal eigenvector corresponding to the steady state number distribution (N_{SS} in our example). This also means that the system is robust, and all sizes of the matrix M will yield a steady state solution. Because all other eigenvalues must have magnitudes less than 1, the corresponding eigenvectors will decay in any superposition state, so the same steady state solution will always be attained regardless of initial state. No change to the numerical values of the parameters in the model will cause the matrix M to violate the conditions of the Perron-Frobenius theorem, hence there will always be a unique steady state no matter how the parameters are altered. This property of stable length control is a robust feature of the system.

This method represents IFT in a flagellum at any fixed length, which determines the size of the state vector and transition matrix. The flagellum grows when motors with cargo reach the tip, and shrinks through a constant, length-independent decay. When the number of motors arriving at the tip times the growth per motor equals the decay in some time interval, the net length change will be zero. Since motors in active transport move at a constant rate, the number of motors injected into active transport is the only factor that controls the number arriving at the tip per second. This value can be expressed as the number of motors in the base multiplied by p, the fraction of motors in the base that get injected into active transport. We can therefore define the critical rate of motors that must arrive at the tip to maintain a steady state length as $G = d/(\delta L * p)$, where d is the decay rate and δL is the growth increment when a single motor reaches the tip. The value of the steady state number density vector N_{SS} in position (2L+1) is the number of motors at the base. This means that when $N_{SS}(2L+1) > G$, there are enough motors at the tip that the flagellum will grow. If $N_{SS}(2L+1) < G$, there are too few motors to counteract the decay, so the flagellum will shrink. This means that when $N_{SS}(2L+1) = G$, the growth factor from motors at the tip perfectly cancels the decay rate. Therefore, when $N_{SS}(2L+1) = G$, the matrix is the right size to encode a flagellum that reaches steady state length.

We can find this matrix by creating transition matrices corresponding to a range of lengths, finding each matrix's principle eigenvalue, and examining the value of the corresponding eigenvector at position (2L+1). Supplemental figure 1B shows the values at this position as a function of L. The horizontal line represents the value of G given by the default parameters in the agent-based model. The matrix that intersects the line at G is the one with the steady state length. The difference between this steady state length and the result from the agent-based model may be explained by the different implementation of avalanching between the models. Note the inverse relationship between injection rate and flagellar length, matching experimental results (16). A possible future direction for this model is making the separation between elements in the matrix correspond to a smaller unit of length, or perhaps a continuous differential equation, allowing us to precisely predict final length. The equilibrium here is stable, reiterating the point that the length would modulate until it reaches steady state. It also means that this system is robust, because any parameter adjustment would retain the stable equilibrium. This model also predicts that the gradient of diffusing motors is linear (Supp. fig. S1C), like in the agent-based model. The benefit of the matrix model in addition to the agent-based model is that it provides an intermediate level of scale that proves stability and robustness, and that it is efficient to vary biochemical parameters and find the steady state solution.



Supplemental figure S1. Markov matrix model

(A) Example of a transition matrix, here with length 4, active transport rate of 0.1, diffusion spread of 0.1, and injection rate of 0.2. The relative sizes of the active transport rate and diffusion rate are roughly equal to the biological parameters used in the agent-based model, but the injection rate is simplified to a length-independent proportion. Based on the active transport and diffusion parameters, this matrix advances a state vector forward in time by 0.05 seconds. (B) Steady-state injection rate as a function of length compared to the value G required for equilibrium. (C) Steady state number density (principal eigenvector) for one set of parameters. x = 1:4 is active transport, x = 5:8 is diffusion, x = 9 is base. Note that the eigenvector can be scaled to an arbitrary magnitude, here it makes sense to normalize it to sum to the number of motors in the system, which we set to 200 for consistency with the agent-based model.