Bidirectionality From Cargo Thermal Fluctuations in Motor-Mediated Transport

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Abstract

Molecular motor proteins serve as an essential component of intracellular transport by generating forces to haul cargoes along cytoskeletal filaments. In some circumstances, two species of motors that are directed oppositely (e.g. kinesin, dynein) can be attached to the same cargo. The resulting net motion is known to be bidirectional, but the mechanism of switching remains unclear. In this work, we propose a mean-field mathematical model of the mechanical interactions of two populations of molecular motors with diffusion of the cargo (thermal fluctuations) as the fundamental noise source. By studying a simplified model, the delayed response of motors to rapid fluctuations in the cargo is quantified, allowing for the reduction of the full model to two “characteristic positions” of each of the motor populations. The system is then found to be “metastable”, switching between two distinct directional transport states, or bidirectional motion. The time to switch between these states is then investigated using WKB analysis in the weak-noise limit.

1. INTRODUCTION

Active transport is an key component of cellular function due to the compartmental nature of cellular machinery. This transport is achieved through the use of molecular motor proteins that undergo a series of conformational changes to walk along cytoskeletal filaments and generate forces to haul cargoes [14]. The transport of a single cargo can often involve two families of motors that are directed oppositely, denoted bidirectional transport [11]. For instance, kinesin, which walks in the positive direction of a microtubule, and dynein, which walks in the negative direction, can be attached to the same cargo. This phenomenon is observed for a variety of cargoes: mRNA particles, virus particles, endosomes, and lipid droplets [12, 18]. Two populations of kinesins oriented oppositely on different microtubule tracks tracks has also been observed [26] and produce the same effect. Although both families of motors are exerting forces on the cargo in opposite directions, the direction of cargo transport is able to switch directions. That is, the cargo spends periods of time with a net positive, negative, and zero velocity (denoted a pause state). This distinct switching suggests a mechanism of cooperation between the motor families that has been explored from both experimental and theoretical perspectives.

The role of external influences in the cooperation mechanism remains unclear. A number of studies have identified regulators of kinesin and dynein [6]. For instance, LIS1 and NudE have been found to modulate dynein’s force production capabilities [24]. In [28], the authors found that the microtubule itself can regulate kinesin force production. However, the necessity of these external regulators for motor coordination in bidirectional transport remains unestablished. The alternative hypothesis relies on the notion that the coordination is a product of the mechanical interactions of the motors with the cargo, denoted a tug-of-war scenario.
The tug-of-war hypothesis has also been investigated from a theoretical and experimental perspective. [25] formulates a mathematical model capable of producing bidirectionality. In the model, the motors share the load equally. This assumption is not always invoked in later mathematical models. For instance, [19] performs stochastic simulations of unequally distributed motors. However, these authors compare the results of the stochastic simulation with experiments and conclude that switching statistics do not match as the number of motors varies. In [29], another mathematical model is proposed where the two motor populations are required to be asymmetric. That is, the two opposing motor populations must have different force generating properties to break symmetry. [21, 22] also provide noteworthy mathematical models, thinking of motor transport as a “rubber-band”-like process. In [2], the authors reexamine the mathematical model of [19] and stress the importance of cargo diffusion for the model to produce the right behavior.

In this work, we present a new tug-of-war model of bidirectional motor-mediated transport. Our proposed model contains fundamentally different essential components than previous work. Specifically, the proposed model is a mean-field model with unequally distributed load. This description therefore is fundamentally different than previous discrete motor, unequal load descriptions and therefore requires a different source of noise to induce switching. After reducing the model, including one that quantifies the delayed response of motors to instantaneous changes in the cargo velocity, we find metastability with two states corresponding to positive and negative net velocities, or bidirectional motion. The noise that drives switching between these two states is due to cargo diffusion (thermal fluctuations), an aspect of this process previously noticed but under-emphasized until recent works [2].

A quantity of interest in validating bidirectional transport models is the reversal or switching time of the system: the time between runs of each direction. In our model, we are able to reduce the question of switching times to a two dimensional escape problem, a classically studied problem. Through the use of WKB analysis in the weak-noise limit, the most probable exit path between the two metastable states can be computed and used to compute the mean switching time. This computation produces a switching time agreeable with experiments [19] and also provides experimental predictions about switching times as temperature changes.

1.1. Model Formulation

Consider a cargo being pulled by two different populations of motors, denoted + and -. Let \( m^{\pm}(x, t) \) be the density of type + or - motors at time \( t \) and stretched from their unstretched position \( x \) units. The + or - labeling of the motor families denotes their preferred directionality. That is, \( m^+ \) corresponds to the density of motors preferring to walk in the positive direction (e.g. kinesin) and \( m^- \) the density of motors preferring to walk in the negative direction (e.g. dynein). The evolution of each motor population is then described by

\[
\frac{\partial m^{\pm}}{\partial t} + \frac{\partial}{\partial x} \left\{ \left[ w^{\pm}(x) - v(t) \right] m^{\pm} \right\} = \left( M^{\pm} - \int_{-\infty}^{\infty} m^{\pm}(x, t) \, dx \right) \omega_{on}^{\pm}(x) - \omega_{off}^{\pm}(x)m^{\pm}(x, t). \tag{1}
\]
Figure 1: A diagram of the mean-field model setup. The quantity, $x$, denoting the distance a motor is stretched is always measured with respect to the orientation of the microtubule.

Although (1) appears as only one equation, $m^+$ and $m^-$ each have their own equation that are structurally identical but may contain different parameters or functional forms. The quantity $x$, describing the distance the motor is stretched from its unstretched position is always measured with respect to the microtubule, even though each motor type walks in a different direction, see Figure 1. This choice of frame of reference is convenient, as it causes the two equations to be structurally identical (as opposed to having to flip the sign of $v$.)

It is worth noting that this PDE has been studied in other contexts, referred to as the Lacker-Peskin PDE [30], which is an extension of the Huxley crossbridge model [15, 17]. In that literature, the particular form of the PDE is derived from the limit of a large number of discrete binding sites. In our context, we consider the case where $M = 1$, producing a probability density description of the position of a motor. However, due to the linearity of the equation in $M$, the description of more than one motor is structurally the same but with $M > 1$.

Before describing, in detail, each term in (1), we state a driving assumption used in the details of several of the functional forms appearing in the equation. The force generated due to the linker stretching is assumed to be Hookean, that is force $\sim kx$, where $k$ is the spring constant or stiffness of the motor linker attachment to the cargo. The force-displacement curve of molecular motors has been studied experimentally [16, 20] and, although not perfectly linear, seems to be well approximated by this assumption.

We now discuss each term of the equation in more detail. Broadly, the motor population can change three ways: motors stepping (walking), binding or unbinding.

1. **stepping:** We assume that the rate of stepping of the motor is dependent on the force exerted on the motor, which is some function of the distance between the motor and the cargo based on the Hookean force assumption previously mentioned. The walking rate
$w(x)$ is therefore position dependent. We take the particular functional form

$$w(x) := -ax + b,$$

where $a > 0$. At $x = 0$, which corresponds to the motor being unstretched, the motor will walk with some velocity $b$. For the $+$ directed motor, for instance, $b > 0$. As the motor walks farther from its unstretched position ($x > 0$), the force exerted on it will cause the velocity to decrease until it eventually stalls at $x_{\text{stall}} := b/a$. If $x < 0$, that is, the cargo is ahead in the direction the motor seeks to walk, the velocity is assumed to be greater as the linker exerts a force in the direction of motion of the motor. If $x > x_{\text{stall}}$, then the force exerted by the linker is greater than the stall force, meaning the motor will move opposite its preferred direction.

This force-velocity curve has been qualitatively observed experimentally [8, 18] in several types of motors, and a (non-linear) version of this functional form has been used in a number of modeling papers [2, 19, 25]. The assumed linearized version allows us to perform analysis on the model.

2. binding: The functional form of the binding term is set to be

$$\omega_{\text{on}}(x) := k_{\text{on}} \delta(x),$$

where $k_{\text{on}}$ is the constant describing the rate of binding of a molecular motor to the cargo. The $\delta(x)$ functional form corresponds to the assumption that motors are initially unstretched ($x = 0$) when they bind, thus only binding at $x = 0$. That is, the motors only bind in a non-force-producing state. This assumption can be relaxed (and will be for later numerical simulations) to a Gaussian approximation of the delta function.

3. unbinding: The unbinding rate of molecular motors has experimentally been found to be related to the force exerted on them [16, 19], however the nature of this dependency is complex and varies from motor to motor. For this reason, we again take the simplest form that still behaves in a way that qualitatively matches experimental results, which is

$$\omega_{\text{off}}(x) = k_{\text{off}} \exp \left\{ k |x| \right\},$$

(2)

where again, the force exerted is assumed to be Hookean ($\sim kx$), $F_D$ is some characteristic force fit to experimental observations, and $k_{\text{off}}$ is the unstretched detachment rate. This can be viewed as a version of Bell’s Law, where Bell’s Law is related to $F_D$. The overall behavior of this function establishes that motors detach at a faster rate the farther they are stretched due to the force exerted on their microtubule binding sites.

In [19], the authors account for the stalling of motors and the catch-bond behavior of dynein by taking a non-monotonic dependence on the force, but this piecewise description is not taken here.

We then can define the average force exerted by each motor population, recalling the assumption of a Hookean force,

$$F^\pm(t) := \int_{-\infty}^{\infty} k^\pm x m^\pm(x, t) \, dx.$$
2. MATERIALS AND METHODS

In this section, we will discuss the analysis performed on the aforementioned model.

2.1. Steady-State Analysis

This time-dependent force, described by (2) is difficult to compute in practice, so we turn our attention to the steady-state force. We will consider the steady state ($\frac{dm^{\pm}}{dt} = 0$ and ) behavior of (1) with some steady-state velocity $\bar{v}$, which leads to the pair of equations for the steady state densities $\bar{m}^{\pm}$

$$\frac{\partial}{\partial x} \left\{ [w^{\pm}(x) - \bar{v}] \bar{m}^{\pm} \right\} = \left( M^{\pm} - \int_{-\infty}^{\infty} \bar{m}^{\pm}(x) \, dx \right) \omega^{\pm}_{on}(x) - \omega^{\pm}_{off}(x) \bar{m}^{\pm}(x).$$

(3)

Exploiting the linearity of (3), along with the partitioning nature of the delta function, (3) can be solved analytically, resulting in a solution with an integrable singularity at the stall position dependent on the velocity $x_{\text{stall}} := \frac{b - \bar{v}}{a}$.

For details of this calculation, see Supplementary Section S1, in the supplementary information. This allows us to define the steady state force exerted by each population of motor

$$\bar{F}^{\pm}(\bar{v}) := \int_{-\infty}^{\infty} k^\pm x \bar{m}(x; \bar{v}) \, dx,$$

(4)

where we are parameterizing this force as a function of the steady state cargo velocity $\bar{v}$ which appears in (3).

We now need an equation governing the cargo velocity, which is determined by the forces exerted on the cargo

$$m\dot{v} + \gamma v = \sqrt{2\gamma k_B T} \xi(t) + \text{forces exerted by motors.}$$

(5)

In (5), $m$ is the mass of the cargo, $\gamma$ is the drag coefficient of the cargo and $\xi(t)$ is the Wiener process due to thermal fluctuations (diffusion) of the cargo. The magnitude of these fluctuations is determined by the fluctuation-dissipation theorem [7].

A perhaps natural choice of the force terms in (5) could be taken to be $\bar{F}^{\pm}(v)$ found in (4), however there is a problem with this choice. As $v$ is changing instantaneously, the position of the cargo is not. The forces exerted by the motors are due to stretching of the linker, and therefore cannot change instantaneously as the velocity changes. Thus, parameterizing the force with time-varying velocity would not produce the physical behavior we desire. For this reason, we turn to a simpler model to understand what to use for the force terms in (5) that accounts for this issue.

In [2], the authors notice that including cargo noise produces this same difficulty: motors should not react instantaneously to velocity and classical models produce results inconsistent
with experimental observations if this is the case. To overcome this issue, the authors hypothesize that the motors respond to a time-windowed-average force, suggesting some “memory” property of the motors. Here, we directly compute a physiological, mechanistic delay stemming from the stepping of the motor, instead of a phenomenological “memory”.

2.1.1. Model Reductions

To understand how the force exerted by a motor varies in time with respect to the cargo velocity, we consider a simple Ornstein-Uhlenbeck equation [7], which can be described by the Langevin equation

\[
dx = \{w(x) - v(t)\} \, dt + \sqrt{2D} \xi(t),
\]

or the corresponding Fokker-Planck equation

\[
\frac{\partial p}{\partial t} = -\frac{\partial}{\partial x} \{[w(x) - v(t)]p\} + D \frac{\partial^2 p}{\partial x^2}.
\]

We interpret this equation in the context of motor-mediated transport by considering \(x\) to be the distance between a motor and the cargo. The cargo is moving with an instantaneous velocity \(v(t)\) and the motor walks with a force (and therefore, position) dependent rate \(w(x)\). The cargo also diffuses under some thermal fluctuations with a diffusion coefficient \(D\).

We consider the mean value of this process, denoted \(\mu\),

\[
\mu := \langle x(t) \rangle.
\]

From the Fokker-Planck equation, we find the relationship describing the temporal evolution of the mean of this process to be (assuming \(w\) is a linear function)

\[
\dot{\mu} = w(\mu) - v(t). \tag{6}
\]

For details of the calculation, see Appendix 5.1.

However, again recalling the assumption of a Hookean force (that is, force \(\sim kx\)), the total force exerted by some population of motors evolving under the process with density \(p(x, t)\) is then

\[
F_{OU} = k \int_{-\infty}^{\infty} xp(x, t) \, dx = k \mu.
\]

In other words, the force exerted can be parameterized by \(\mu\), where \(\mu\) “tracks” the velocity through (6). This resolves the aforementioned issue about the force changing instantaneously. Now, the force tracks, with some delay as determined by (6), the velocity and evolves continuously.

Using the observation in the previous section, we now take our model for the velocity evolution to be of the form

\[
m\ddot{v} + \gamma \dot{v} = \bar{F}(\mu) + \sqrt{2\gamma k_B T} \xi(t), \quad \dot{\mu} = w(\mu) - v. \tag{7}
\]

In other words, \(v\) evolves with the forces exerted on it, but the force exerted by the motors is not directly determined by \(v\) but rather some parameter \(\mu\) which tracks \(v\) with a delay. We
can think of \( \mu \) as a “characteristic distance”. That is, the force exerted by the motors \( \hat{F}(\mu) \) is effectively the force exerted by a population of motors with mean position \( \mu \), staying in the spirit of the mean-field model. We discuss the specific choice of \( \hat{F} \) later.

The parameter regime we are considering deals with cargo with negligible mass, thus suggesting we are in a viscous or near-viscous regime. Exploiting this fact, we can perform an adiabatic (quasi-steady state) reduction on (7) to eliminate \( v \). For details of this calculation, see Supplementary Section S3. The result of performing this reduction is

\[
\dot{\mu} = w(\mu) - \frac{\hat{F}(\mu)}{\gamma} + \sqrt{\frac{2k_B T}{\gamma}} \xi(t),
\]

or equivalently, in Fokker-Planck form

\[
\frac{\partial p}{\partial t} = -\frac{\partial}{\partial \mu} \left\{ w(\mu) - \frac{1}{\gamma} F(\mu) \right\} + \frac{k_B T}{\gamma} \frac{\partial^2 p}{\partial \mu^2}. \tag{8}
\]

One important note from the calculation detailed in Supplementary Section S3 is that although \( v \) is eliminated from the system, \( v \) relaxes quickly to a Gaussian centered around \( \hat{v} \sim \hat{F}(\mu)/\gamma \), thus the value of \( \mu \) directly determines the (mean) velocity.

Combining all of the previous observations, we now propose the full model. It should first be noted that in the derivation of (8), only one motor population was considered, but in bidirectional transport, there are two populations evolving separately, resulting in two equations with identical structure but different parameters. From this, we get the full model (in Fokker-Planck form)

\[
\frac{\partial p}{\partial t} = -\frac{\partial}{\partial \mu_1} \left\{ \left[ a_1 \mu_1 + b_1 - \frac{1}{\gamma} \left\{ \hat{F}_1(\mu_1) + \hat{F}_2(\mu_2) \right\} \right] p \right\} + \frac{k_B T}{\gamma} \frac{\partial^2 p}{\partial \mu_1^2} \\
- \frac{\partial}{\partial \mu_2} \left\{ \left[ a_2 \mu_2 + b_2 - \frac{1}{\gamma} \left\{ \hat{F}_1(\mu_1) + \hat{F}_2(\mu_2) \right\} \right] p \right\} + \frac{k_B T}{\gamma} \frac{\partial^2 p}{\partial \mu_2^2}, \tag{9}
\]

which we abbreviate as

\[
\frac{\partial p}{\partial t} = -\nabla \cdot \{ b(\mu)p \} + \frac{k_B T}{\gamma} \nabla^2 p.
\]

Note that we have switched the two populations to labels \( j = 1, 2 \) instead of \(+/-\) for notational convenience. We also recall that \( w(x) = -ax + b \) and that the total force exerted by the motors is the sum of the force exerted by each population.

One detail yet to be resolved is the choice of \( \hat{F}_j(\mu) \). We take the force exerted by the motors to be the steady state force generated, described by (4) such that

\[
\hat{F}_j(\mu) = \tilde{F}_j(-a_j \mu_j + b_j). \tag{10}
\]

We justify this by observing that \( \tilde{F} \) was computed for motors equilibrated for a particular constant \( \tilde{v} \), which we can think of this as when \( \dot{\mu} = 0 \) (that is, the mean position of the population is not changing), and therefore

\[
\dot{\mu} = 0 = -a \mu + b - \tilde{v} \implies \tilde{v} = -a \mu + b,
\]

meaning we associate \(-a_j \mu_j + b_j\) with \( \tilde{v} \) to obtain (10).
Table 1: “Typical” motor values used for both populations of motors in the symmetric case of the mean field model.

3. RESULTS AND DISCUSSION

3.1. Metastable Behavior

To emphasize the ability of this model to produce bidirectional motion without asymmetry between the motor populations, we take the parameters describing each of the populations to be the same, described in Table 1. These parameters are chosen as physiologically reasonable parameters in the range of reported values of both kinesin and dynein.

Although the motors have identical parameter values, the second motor population still has motion directed oppositely to the first. Thus, $b_2 = -b_1$ in the walking rate function $w(x)$. An immediate consequence of this choice is that $F_2(\mu_2) = -F_1(-\mu_1)$. Thus, we abbreviate this as $F(\mu) := F_1(\mu)$.

Figure 2 shows two example time simulations of the Langevin description of the full system...
described by (9). Although the noise is strong enough that the time dependent simulation on its own is difficult to interpret, a histogram of the attained values reveals a curious behavior, namely metastability. That is, \( \mu_1, \mu_2 \) each have a bimodal distribution: there are two states in the \((\mu_1, \mu_2)\) plane that that are “metastable”, meaning the system spends most of its time near these two points. If we reduce the thermal noise, by fixing \( k_BT/\gamma \) to be a smaller value, seen in Figure 2, this behavior becomes more pronounced and apparent: the system switches between two configurations.

![Diagram showing the most probable exit path from \( S_1 \) to \( S_2 \) as well as the action along it. The deterministic flow field \( b(\mu) \) is also shown for comparison.](image)

**Figure 3:** A depiction of the most probable exit path from \( S_1 \) to \( S_2 \) as well as the action along it. The deterministic flow field \( b(\mu) \) is also shown for comparison.

To see the underlying mechanism behind this, we study the deterministic flow field, which can be seen in Figure 3. The deterministic system is bistable with two stable equilibria (denoted \( S_1, S_2 \)) separated by a separatrix containing a hyperbolic (saddle) point (denoted \( H \)). This is verified by computing the eigenvalues of the Jacobian at \( H \). Recalling that the deterministic drift is described by

\[
b(\mu_1, \mu_2) = \begin{bmatrix} b_1(\mu_1, \mu_2) \\ b_2(\mu_1, \mu_2) \end{bmatrix} = \begin{bmatrix} -a\mu_1 + b - \frac{1}{\gamma} \{ \hat{F}_1(\mu_1) + \hat{F}_2(\mu_2) \} \\ -a\mu_2 - b - \frac{1}{\gamma} \{ \hat{F}_1(\mu_1) + \hat{F}_2(\mu_2) \} \end{bmatrix},
\]

and that \( F(\mu) = \hat{F}_1(\mu) \) and \( \hat{F}_2(\mu_2) = -F(-\mu) \), we find the eigenvalues of the Jacobian are

\[
\lambda_1 = -a, \quad \lambda_2 = -a - \frac{1}{\gamma} \{ F'(\mu_1) + F'(-\mu_2) \}.
\]

From this, we see that we always have one negative eigenvalue and the sign of the second eigenvalue depends on the derivative of \( F(\mu) \).

We can also find one of the equilibria directly by noting that if \( b_1(\mu) = b_2(\mu) = 0 \) and we can subtract these two to find that equilibria must occur on the line

\[
0 = -a(\mu_1 + \mu_2) + 2b.
\]
The hyperbolic point, \( H \), is then, due to symmetry, \( H = (\mu_1, \mu_2) = (b/a, -b/a) \).

The significance of these steady states can be understood by recalling that the velocity of the cargo is Gaussian centered around \( \hat{v} \) where

\[
\hat{v}(\mu_1, \mu_2) = \sum F/\gamma = \frac{1}{\gamma} [F_1(\mu_1) + F_2(\mu_2)].
\]  

Thus, in Figure 4 we show a contour plot of \( \hat{v}(\mu_1, \mu_2) \) as described by (13). In this figure, we see the two stable equilibria correspond to a negative and positive \( \hat{v} \), the mean velocity. The system switches between these two states and therefore demonstrates bidirectional motion. It should also be noted the actual value of the mean velocity is physiologically reasonable, agreeing with experimental values [1, 27].

![Figure 4: A contour plot of the center of the Gaussian describing \( v \), which is centered at \( \hat{v} = [F_1(\mu_1) + F_2(\mu_2)]/\gamma \). The two metastable states correspond to a positive and negative mean velocity and the hyperbolic “pause” state corresponds to a zero mean velocity.](image)

The \( v \sim 0 \) result at the hyperbolic point can be computed directly by recalling that when the velocity of the cargo is equal to the motors unstretched velocity, no displacement occurs and therefore no force generation, resulting in

\[
\tilde{F}_+(b) = 0 \implies F(b/a) = 0 \implies b(S) = 0,
\]

and recalling the form of \( b \) found in (11), we conclude that \( \hat{v} = 0 \) at the hyperbolic point \( H \). The value of \( \hat{v} \) at the other fixed points of the deterministic system must be computed numerically. This hyperbolic point can be interpreted as the “pause” state often observed in motor-mediated bidirectional transport [11].

### 3.2. Weak-Noise Escape Problem

From the previous section, we see that the system switches between a mean positive and mean negative cargo velocity state, or bidirectional motion. A quantity of immediate interest is the
reversal (switching) time between these two states, which can be compared to experimental 
values found in [19].

In this section, we seek to compute the switching time between these two states by taking 
a weak-noise limit. Although there is no physical motivation for the noise being weak, the 
approximation holds reasonably well for physiologically reasonable noise levels.

To do so, first consider non-dimensionalizing \((9)\) under the transformation 
\[
s = ta, \quad z = \mu a/b,
\]
which results in the system
\[
\begin{align*}
\frac{\partial p}{\partial s} &= -\partial_{z_1} \left\{ -z_1 + 1 - \frac{1}{\gamma b} \left[ F \left( z_1 \frac{a}{b} \right) - F \left( -z_2 \frac{a}{b} \right) \right] p \right\} + \frac{k_B T a}{b^2} \partial_{z_1} p \\
&\quad - \partial_{z_2} \left\{ -z_2 - 1 - \frac{1}{\gamma b} \left[ F \left( z_1 \frac{a}{b} \right) - F \left( -z_2 \frac{a}{b} \right) \right] p \right\} + \frac{k_B T a}{b^2} \partial_{z_2} p,
\end{align*}
\]
which we abbreviate as
\[
\frac{\partial p}{\partial s} = \mathbb{L} p := -\nabla_z \cdot \{ B(z) p \} + \frac{\varepsilon}{2} \nabla^2_z p.
\] (14)

The switching time of \((14)\) can now be computed in the weak noise limit as \(\varepsilon \to 0\) using 
Wentzell-Freidlin theory [5]. Although the general components of the calculations are described 
here, the derivation of these and all of the details can be found in [23] and were originally de-
developed in that same work.

It should be noted that \(B(z)\) is not a conservative vector field satisfying detailed balance. That 
is, \(B\) is not-curl free \((\nabla \times B \neq 0)\) or the gradient of some scalar potential (that is, there is no \(V\) 
such that \(B = -\nabla V\)). If this were the case, the switching time calculation reduces considerably 
in difficulty.

Ultimately, the calculation of the mean first passage time then reduces to the study of the prin-
ciple (largest non-zero) eigenvalue of the operator \(\mathbb{L}\) described by \((14)\). Due to the exponentially 
small nature of this eigenvalue, it can be approximated by solving \(\mathbb{L} p = 0\), that is, finding the 
quasistationary solution of the Fokker-Planck equation \((14)\). However, the appropriate boundary 
conditions for the leading eigenvalue cannot be satisfied by the quasistationary distribution, 
so a matched asymptotic analysis is performed to correct this. The relationship between 
these ideas is more explicitly described in Supplementary Section S4.

To find the quasistationary solution, a large deviation principle [3] suggests the use of the WKB 
ansatz
\[
p(z) = K(z) \exp \left\{ -\Phi(z)/\varepsilon \right\}.
\] (15)
\(\Phi(z)\), found in \((15)\), is called the \textit{quasipotential} and serves as an effective energy barrier. \(K(z)\) is 
referred to as the exponential \textit{prefactor}. Inserting this ansatz into \((14)\) yields, to leading order in \(\varepsilon\):
\[
\mathcal{H}(z, \nabla \Phi) = 0, \quad \mathcal{H}(z, p) := B(z) \cdot p + \frac{1}{2} [p \cdot p].
\] (16)
That is, to leading order, we have a Hamilton-Jacobi equation, where \(\mathcal{H}\) is referred to as the 
Wentzell-Freidlin Hamiltonian [5] or the “energy” of the system. The quantity \(p\) is referred to as 
the conjugate momentum and satisfies \(p = \nabla \Phi\). An immediate consequence of the Hamiltonian
structure is the equivalence of (16) to Hamilton’s equations:

\[ \dot{z} = \nabla_p H, \quad \dot{p} = -\nabla_z H, \]

where we now consider \( z(t), p(t) \), that is, trajectories parameterized by \( t \) and note that dots correspond to derivatives with respect to this parameterization. These parameterized solutions can be thought of as “zero energy” trajectories of the system. Associated with every Hamiltonian is also a Lagrangian:

\[ L(z, \dot{z}) = \frac{1}{2} ||\dot{z} - B(z)||^2, \]

which allows us to define the classical action

\[ S_T[z] := \int_0^T L(z, \dot{z}) \, dt. \]

This then provides another interpretation of the trajectories satisfying Hamilton’s equations, which minimize the action. In fact, if we consider trajectories starting at \( z^* \), the stable fixed point (say, corresponding to \( S_1 \) in original coordinates), we then have the formulation of the quasipotential

\[ \Phi(z) = \inf_{T>0, y:[0,T] \to \Omega, y(0)=z^*, y(T)=z} S_T[y], \quad (17) \]

where \( \Omega \) is the basin of attraction of \( z^* \) in the deterministic flow \( B(z) \).

In other words, \( \Phi(z) \), the quasipotential, measures how “difficult” it is for a particle to travel from the stable fixed point to the point \( z \). These trajectories could also be found by using the method of characteristics on the Hamilton-Jacobi equation (16).

We assume that the quasipotential has a minimum along the separatrix at the hyperbolic point, meaning the probability of exit through the separatrix is largest at this point. We then seek the most probable exit path (MPEP), defined to be the action minimizing trajectory from \( z^* \) to the hyperbolic point. This is not necessarily the spatial “mean path” as we see later, but still contributes the greatest to the mean escape time.

One technique for computing the most probable exit path is to integrate Hamilton’s equations along trajectories and do a ray search for the most probable exit path. This technique was successfully performed in another paper of bidirectional motion \([9, 10]\) however this technique did not work in this case for numerical reasons, specifically due to the stiffness of the integration.

Thus, an alternate technique was utilized to find the most probable exit path: the geometric minimum action method \([13]\). This technique exploits the idea that the action can be minimized independent of parameterization, leading to a simpler minimization where the curve is parameterized with respect to arc-length. More details of this method can be found in Supplementary Section S5.

The result of performing the geometric minimum action technique can be seen in Figure 3. We see that the most probable exit path is tangent to the separatrix, and then relaxes quickly to second equilibrium. This feature is confirmed from the theory established in \([23]\), which states
that a key quantity is
\[ \mu := \frac{|\lambda_1|}{\lambda_2}, \]
where \( \lambda_1, \lambda_2 \) are the two eigenvalues of the Jacobian of the deterministic flow at the hyperbolic point. We see from (12) that necessarily, if \( \lambda_2 \) is positive then \( \mu < 1 \), which is confirmed numerically. In this \( \mu < 1 \) case, the approach to the hyperbolic point is tangent to the separatrix. Interestingly, in this case, the distribution of exits is actually skewed to be only in one direction from the hyperbolic point along the separatrix. Thus, even though the most probable exit path is tangent to the separatrix, the actual distribution of exit locations along the separatrix is skewed away from \( H \).

From the geometric minimum action method, we are able to recover the most probable exit path and the action along this path. This provides us with \( \Phi, x, p \), but to compute the mean exit time, we need knowledge of the exponential prefactor \( K \). Plugging in the WKB ansatz into the stationary equation, to second order, \( K \) satisfies
\[ \frac{\dot{K}}{K} = -\left[ \nabla \cdot B + \frac{1}{2} \nabla^2 \Phi \right]. \tag{18} \]

We now have a transport equation for \( K \) but this requires knowledge of the Hessian of the quasipotential. Let \( \Phi_{,ij} := \partial_i \partial_j \Phi \), then it can be shown that the Hessian satisfies the Riccati equation
\[ \dot{\Phi}_{,ij} = -\frac{\partial^2 \mathcal{H}}{\partial p_k \partial p_l} \Phi_{,ik} \Phi_{,jl} - \frac{\partial^2 \mathcal{H}}{\partial x_j \partial p_k} \Phi_{,ik} - \frac{\partial^2 \mathcal{H}}{\partial x_i \partial p_k} \Phi_{,jk} - \frac{\partial^2 \mathcal{H}}{\partial x_i \partial x_j}. \tag{19} \]

If we consider trajectories emanating from the stable fixed point \( z^* \) then we must take \( t \to -\infty \), which, when applying to, also noting that at the equilibria \( p = 0 \), denoting \( Z = [\Phi_{,ij}^0] \), our matrix of initial Hessian values, and \( B = [B_{i,j}] \), the linearized deterministic flow, we get the initial condition matrix equation
\[ ZZ + ZB + B^T Z = 0, \]
which if we take \( C = Z^{-1} \), yields the easier to solve algebraic Riccati equation
\[ I + BC + CB^T = 0. \]

Thus, we can integrate (18) and (19) along our most probable exit path obtained by the geometric minimum action method. One technical note is that the geometric minimum action method provides \( z(\alpha), p(\alpha) \), that is, a parameterization with respect to arc-length \( \alpha \), but the \( t \) parameterization can be recovered (see Supplementary Section S5 for details) and is used for the integration.

If we let \( \hat{z} = (\hat{z}_1, \hat{z}_2) \) be the coordinate system rotated by \( \pi/4 \) and shifted so that the hyperbolic point is the origin and the separatrix becomes the line \( \hat{z}_1 = 0 \) (which does not change the result since the diffusion tensor is still \( \sigma \sigma^T = I \)) then \([23]\) and briefly \([9]\) derive the result for the mean first passage time (\( \bar{\tau} \)) in the \( \mu < 1 \) case to be
\[ 2\bar{\tau}^{-1} = \lim_{z \to 0} \text{along MPEP} \frac{\mu \hat{z}_2}{2\pi \hat{z}_1} K(z) \sqrt{\det \Phi_{,ij}(z^*)} e^{-\Phi(0)/\varepsilon}, \tag{20} \]
where again we emphasize the coordinate change so that the hyperbolic point is now the origin. A technical note is that the exponential prefactor actually tends to 0 as the path approaches the hyperbolic point, but the limit involved in (20) is finite and non-zero.

We perform Monte Carlo simulations of the Langevin description of the system and keep track of the distribution of switching times for various values of \( \varepsilon \). We then can compute the WKB predicted switching time, which is parameterized by \( \varepsilon \) and compare the result of these two procedures, as seen in Figure 5, which plots these predictions against \( \tau \), the switching time in original units of seconds. Although the agreement between the two is not perfect, it is apparent the weak-noise approximation provides an accurate prediction for a range of \( \varepsilon \). In fact, the prediction seems more accurate for larger values of \( \varepsilon \) even though the computation is done in the small \( \varepsilon \) limit.

\[
2k_B T/\gamma \text{ [nm}^2/\text{s]} \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \quad 12 \quad 14
\]

\[
\text{log}(\tau) \quad \text{predicted} \quad \text{monte carlo mean}
\]

Figure 5: A violin plot comparing the distribution of switching times from Monte Carlo simulations (the violins, means shown) with the prediction from the weak noise WKB analysis.

For the physiological parameter values used in the model, at standard \( k_B T \), the mean switching time is predicted to be \( \tau \sim 0.5 \) [s], which agrees with experimental values in [19]. Since the noise of the system is driven by thermal fluctuations, the \( \varepsilon \) parameterization of the switching time of the system can be interpreted as a temperature parameterization of the switching time. Thus, on the top axis of Figure 5, the noise strength in original variables is used, showing that we have an experimental prediction for the reversal time as a function of temperature.
4. CONCLUSION & DISCUSSION

In this work, we have proposed a mean-field, unequally distributed load description of motor-mediated transport. To understand the behavior of this model, we perform a series of reductions. The first, inspired by a simple Ornstein-Uhlenbeck process, quantifies the delay in which motors are able to respond to instantaneous changes in the cargo velocity. Secondly, we use the small mass of the cargo to perform an adiabatic (quasi-steady state) reduction of the system. The result is a system of two stochastic differential equations describing $\mu_j$, a “characteristic” position for each population of motors. This resulting stochastic system is observed to be “metastable”, switching between two distinct states because of noise. From the adiabatic reduction, we associate each of these states with a positive and negative mean cargo velocity, thus bidirectional transport. To quantify the reversal time of the system, we take the weak-noise limit of the system and use WKB analysis to compute the most probable exit path. Asymptotic theory then provides an estimate of the reversal time which agrees well with Monte Carlo simulations and experimental observations.

The Ornstein-Uhlenbeck analysis for quantifying the ability of a motor to react to instantaneous changes in cargo velocity is of interest in other recent works [2]. In this paper, the authors hypothesize a “motor memory” and conclude that models only agree with experimental values appropriately if the motors react to a windowed-time-average velocity. In our work, we have quantified this “memory” directly from the physiology of the motor. However, our analysis was only performed for a single motor and was assumed to hold for a population. Thus, quantifying this reaction for a whole population is still desirable.

In [2], the authors also cite the importance of cargo diffusion in models producing results that match experimental values. In our work, we have further illustrated the importance of cargo diffusion by illustrating its ability to produce qualitative changes in motor-mediated transport. Specifically, the fundamental noise driving switching in our model is cargo diffusion, unlike previous unequally distributed load models which depended on a discrete motor description. This raises the possibility of the importance of diffusion in other aspects of motor-mediated transport.

Thus, we have illustrated that common features of previous work: discreteness of the motors, asymmetry of motor populations, equally distributed loads are not necessary to produce a physiologically reasonable model of bidirectional motor transport. This raises uncertainty of which key ingredients may be essential for tug-of-war, making it even more difficult to compare to the alternative regulatory hypothesis of bidirectionality. However, in our work, we have provided an experimental prediction of the reversal time as a function of the system temperature. If indeed thermal noise is the driver of this switching, then agreement with this experiment would support this claim. An immediate issue is that adjusting the system temperature would also change properties of the motors (that is, their stepping or unbinding, binding rates), but it seems feasible this adjustment is negligible compared to the magnitude in which the thermal fluctuations would change.
5. APPENDIX

5.1. Ornstein-Uhlenbeck Mean Evolution

In this section, we show that if the advection term of an Ornstein-Uhlenbeck has a time dependence, a differential equation can be obtained for the mean of the process, demonstrating an effective delay.

Consider a Fokker-Planck equation of the form
\[ \partial_t p = -\partial_x \left[ \{w(x) - v(t)\} p \right] + D \partial_{xx} p. \tag{21} \]

Denote \( \mu(t) \) to be the mean of the process, that is \( \mu = \langle p \rangle \). Then, we have:
\[ \dot{\mu} = \frac{d}{dt} \int_{-\infty}^{\infty} xp(x,t) \, dx = \int_{-\infty}^{\infty} x \partial_t p \, dx. \]

However, we can use (21) to find that
\[ \dot{\mu} = -\int_{-\infty}^{\infty} x \partial_x \left[ \{w(x) - v(t)\} p \right] \, dx + \int_{-\infty}^{\infty} xD \partial_{xx} p \, dx, \]
which, after integration by parts, yields
\[ \dot{\mu} = \langle w(x) \rangle - v. \]

Jensen’s inequality states that for a convex \( w \)
\[ \langle w(x) \rangle \geq w(\langle x \rangle), \]
however, if we assume \( w(x) \) is linear (as we have done in the model), then Jensen’s inequality attains equality and the result is
\[ \dot{\mu} = w(\mu) - v(t). \]

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at \texttt{http://www.biophysj.org}. The supplement contains four sections:

S1. a derivation of an analytical expression for the steady-state motor density

S2. force-velocity curves as different parameter values are adjusted

S3. a WKB theory supplement to make the computation performed in the paper more explicit

S4. additional details on the geometric minimum action method, the numerical scheme used to compute the most probable exit path.
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S1. Steady-State Force Density

In this section, we construct an analytical solution to the steady-state mean field equation (3) with the particular choice of functional forms described in the chapter. Thus, we are looking at equations of the form

$$\partial_x \left\{ \left( w(x) - v \right) m \right\} + k_{\text{off}} e^{k|x|/FD} m = \left\{ M - \int_{-\infty}^{\infty} m(x) \, dx \right\} k_{\text{on}} \delta(x).$$

The first observation that can be made is: due to the linearity of this equation, we can reduce it to the study of the simpler equation

$$\partial_x \left\{ \left( w(x) - v \right) u \right\} + k_{\text{off}} e^{k|x|/FD} u = k_{\text{on}} \delta(x), \quad (S1)$$

where \( m(x) \), the original solution can be recovered via the relationship

$$m(x) = \frac{M}{1 + U} u(x), \quad U := \int_{-\infty}^{\infty} u(x) \, dx.$$  

We now divide everything through by \( k_{\text{off}} \) in \( (S1) \) and recall \( w(x) = -ax + b \). Denote the rescaled variables \( \cdot / k_{\text{off}} \) by \( \tilde{\cdot} \) and also abbreviate \( k/FD = \alpha \), yielding

$$\partial_x \left\{ (-\tilde{a}x + \tilde{b} - \tilde{v}) u \right\} + \exp\{\alpha|x|\} u = \tilde{k}\delta(x). \quad (S2)$$

We can now split this into two scenarios: to the left of \( x = 0 \) and to the right:

$$\begin{cases} 
\partial_x \left\{ (-\tilde{a}x + \tilde{b} - \tilde{v}) u_L \right\} + \exp\{-\alpha x\} u_L = 0 & \text{for } x < 0, \\
\partial_x \left\{ (-\tilde{a}x + \tilde{b} - \tilde{v}) u_R \right\} + \exp\{\alpha x\} u_R = 0 & \text{for } x > 0.
\end{cases} \quad (S3)$$

These two equations must satisfy a matching condition at \( x = 0 \), so consider integrating \( (S2) \) a tiny window around \( x = 0 \) from \( -\varepsilon \) to \( \varepsilon \), yielding

$$\int_{-\varepsilon}^{\varepsilon} \partial_x \left\{ (-\tilde{a}x + \tilde{b} - \tilde{v}) u \right\} + \exp\{\alpha|x|\} u = (b - v) [u_R(0) - u_L(0)] = \int_{-\varepsilon}^{\varepsilon} \tilde{k}\delta(x) \, dx = \tilde{k}.$$  

In other words, we have the matching condition

$$(b - v) [u_R(0) - u_L(0)] = \tilde{k}.$$
Integrating (S3), we find

\[
\begin{align*}
    u_L(x) &= \frac{\alpha_L}{\tilde{a}x - \tilde{b} + \tilde{v}} \exp \left\{ \frac{1}{\tilde{a}} \exp \left( \frac{-\tilde{b} + \tilde{v})\alpha}{a} \right) \text{Ei} \left( \frac{-(-\tilde{b} + \tilde{v} + \tilde{a}x)}{\tilde{a}} \right) \right\}, \\
    u_R(x) &= \frac{\alpha_R}{\tilde{a}x - \tilde{b} + \tilde{v}} \exp \left\{ \frac{1}{\tilde{a}} \exp \left( \frac{-\tilde{b} - \tilde{v})\alpha}{a} \right) \text{Ei} \left( \frac{-(-\tilde{b} - \tilde{v} + \tilde{a}x)}{\tilde{a}} \right) \right\},
\end{align*}
\]

where \(\alpha_R, \alpha_L\) are unknown constants and \(\text{Ei}\) is the exponential integral. The matching of these two can be simplified by the realization: only one of \(u_L, u_R\) is non-zero.

That is, if \(-\tilde{a}x + \tilde{b} - v > 0\), then the advection is rightward (only starting from \(x = 0\)) and therefore \(u_L = 0\). Similarly, if the advection is leftward then \(u_R = 0\) necessarily. It should also be noted that (S4) demonstrate the integrable singularity at \(x^* = \frac{-\tilde{a} + \tilde{b}}{\tilde{a}}\), beyond this point, the solution is also necessarily zero. Thus, the solution reduces to either the interval \([0, x^*]\) or \([x^*, 0]\) depending on the sign of \(x^*\), or really, if \(b > v\).

Thus, if \(b > v\), then \(x^* > 0\) and \(u_L < 0\) and if \(b < v\) then \(x^* < 0\) and \(u_R = 0\). Thus, if \(b > v\), then our matching condition provides us \(\alpha_R\):

\[
\alpha_R = -\tilde{k} \exp \left\{ \frac{1}{\tilde{a}} \exp \left( \frac{(\tilde{b} - \tilde{v})\alpha}{a} \right) \text{Ei} \left( \frac{(\tilde{b} + \tilde{v})\alpha}{\tilde{a}} \right) \right\}.
\]

Similarly, in the case that \(b < v\), we have

\[
\alpha_L = \tilde{k} \exp \left\{ \frac{1}{\tilde{a}} \exp \left( \frac{(\tilde{b} + \tilde{v})\alpha}{\tilde{a}} \right) \text{Ei} \left( \frac{(\tilde{b} - \tilde{v})\alpha}{a} \right) \right\}.
\]

Thus, we have constructed all components of the analytical solution to the original steady state equation.
S2. Force-Velocity Curves

In this section, we plot the steady state force-velocity curves described by (4). In these plots, the parameter values are taken to be those described by Table 1 except for one parameter (shown in the legend), which is adjusted over a range of values.

Figure S1: Plots of the steady state force distribution $\tilde{F}$ parameterized by the velocity of the cargo for different parameter values.
### S3. Adiabatic Reduction Details

In this section, we perform an adiabatic reduction of \((7)\), which, recalling the form of \(w(x)\) and using \(F(x) = kx\) for the sake of illustration, yields

\[
m\dot{v} + \gamma v = kx + \sqrt{2\gamma k_B T} \xi(t), \quad \dot{x} = ax + b - v,
\]

which is equivalent to the Fokker-Planck equation

\[
\frac{\partial p}{\partial t} = -\frac{\partial}{\partial y} \{ (ax + b - v)p \} - \frac{1}{m} \frac{\partial}{\partial v} \{ (kx - \gamma v)p \} + \frac{k_B T \gamma}{m^2} \frac{\partial^2 p}{\partial v^2}.
\]

\[(S5)\]

We first perform a non-dimensionalization. Let \(y = x/x_0, \tau = t/t_0\), which provides a scaling on the velocity \(u = vt_0/x_0\), all of which are dimensionless, where we particularly take \(t_0 = \gamma/k\), and set \(\gamma t_0/m = 1/\varepsilon\), which gives us that \(\gamma^2/km = 1/\varepsilon\). We can then also set the last term \(\gamma k_B T t_0^2/m^2 x_0^2 = 1/\varepsilon\), which gives us that \(x_0 = \sqrt{k_B T \gamma^2/mk^2}\). Then, \((S5)\) becomes

\[
\frac{\partial p}{\partial \tau} = -\frac{\partial}{\partial y} \{ (\alpha y + \beta - u)p \} + \frac{1}{\varepsilon} \frac{\partial}{\partial u} \left\{ (u - y)p + \frac{\partial p}{\partial u} \right\},
\]

which we denote

\[
\frac{\partial p}{\partial \tau} = \frac{1}{\varepsilon} \mathbb{L}_1 p + \mathbb{L}_2 p.
\]

Note, the null-space of the fast operator, \(\mathbb{L}_1\) is not the same as the classical Brownian due to a different choice of \(\varepsilon\).

Now, if \(\phi \in \text{null}(\mathbb{L}_1)\), then it satisfies the following differential equation:

\[
\frac{\partial \phi}{\partial y} + (u - y)\phi = 0,
\]

which has a solution

\[
\phi(u) = \frac{1}{\sqrt{2\pi}} \exp\{- (u - y)^2/2\}.
\]

Define the projection operator \(\mathbb{P}\) as

\[
\mathbb{P}f := \phi(u, y) \int_{-\infty}^{\infty} f(u, y) \, du, \quad \mathbb{Q} := 1 - \mathbb{P}.
\]

We then split our solution \(p\) into the part in the null-space of the fast operator and otherwise. That is,

\[
p = \mathbb{P}p + \mathbb{Q}p = v + w,
\]

where we take \(v\) to be of the form \(v = f(y, t)\phi(u, y)\), as it is in the null space of \(\mathbb{L}_1\), and \(f\) is some unknown amplitude.

We first consider applying \(\mathbb{L}_2\) to \(v\) for later calculations

\[
\mathbb{L}_2 v = \mathbb{L}_2 \mathbb{P} p = -\frac{\partial}{\partial y} \{ (\alpha y + \beta - u) f(y) \phi(u, y) \}.
\]
Now, applying $\mathbb{P}$ to this result yields
\[
\mathbb{P}\mathbb{L}_2 \mathbb{P} p = - \frac{\partial}{\partial y} \{ (\alpha y + \beta - y) f \} \phi(u, y).
\]

Next, we consider applying $\mathbb{P}$ and $\mathbb{Q}$ to the Fokker-Planck equation to yield the differential equation
\[
\mathbb{P} \left( \frac{\partial p}{\partial \tau} \right) = \frac{\partial v}{\partial \tau} = \mathbb{P} \left( \frac{1}{\varepsilon} \mathbb{L}_1 + \mathbb{L}_2 \right) (v + w) = \mathbb{P}\mathbb{L}_2 v + \mathbb{P}\mathbb{L}_2 w = - \frac{\partial}{\partial y} \{ (\alpha y + \beta - y) f \} \phi(u, y).
\]
based on the first calculation and the fact that $\mathbb{P}\mathbb{L}_1 = 0$ by construction. Next, we have
\[
\mathbb{Q} \left( \frac{\partial p}{\partial \tau} \right) = \frac{\partial w}{\partial \tau} = \mathbb{Q} \left( \frac{1}{\varepsilon} \mathbb{L}_1 + \mathbb{L}_2 \right) (v + w)
= \frac{1}{\varepsilon} \mathbb{L}_1 w + \mathbb{Q}\mathbb{L}_2 (v + w)
= \frac{1}{\varepsilon} \mathbb{L}_1 w + \mathbb{L}_2 v + \mathbb{L}_2 w - \mathbb{P}\mathbb{L}_2 v - \mathbb{P}\mathbb{L}_2 w.
\]
Noting that, again $\mathbb{P}\mathbb{L}_1 = 0$ and $\mathbb{L}_1 v = 0$ by construction. We now take $w$ to be in quasi-steady state, meaning it must satisfy
\[
\frac{1}{\varepsilon} \mathbb{L}_1 w = - \mathbb{L}_2 v + \mathbb{P}\mathbb{L}_2 v,
\]
which, when using the definitions of these operators yields
\[
\frac{1}{\varepsilon} \frac{\partial}{\partial u} \left\{ (u - y) w + \frac{\partial w}{\partial u} \right\} = \frac{\partial}{\partial y} \{ (\alpha y + \beta - u) f(y) \phi(u, y) \} - \frac{\partial}{\partial y} \{ (\alpha y + \beta - y) f \} \phi(u, y).
\]
We integrate once with respect to $u$ to get rid of a derivative on the left hand side, finding that
\[
\frac{1}{\varepsilon} \left\{ (u - y) w + \frac{\partial w}{\partial u} \right\} = \phi \left\{ f(y)(u - \alpha y - \beta) + f' \right\}.
\]
and therefore, using an integrating factor
\[
w = \varepsilon \frac{2}{\phi u} \left\{ f(y)(u - 2\alpha y - 2\beta) + 2f'(y) \right\}.
\]
Now, using this form of $w$, we must compute $\mathbb{P}\mathbb{L}_2 w$, since that is the term in the $\partial v/\partial t$ equation. First, applying $\mathbb{L}_2$, by definition:
\[
\mathbb{L}_2 w = - \frac{\partial}{\partial y} \{ (\alpha y + \beta - u) w(u, y) \}.
\]
and now projecting yields
\[
\mathbb{P}\mathbb{L}_2 w = \varepsilon \left[ f(y) + y f'(y) + f''(y) \right] \phi(u).
\]
Thus, our differential equation for $v$ is
\[
\frac{\partial v}{\partial \tau} = - \varepsilon \frac{\partial}{\partial y} \{ (\alpha y + \beta) f \} + \mathbb{P}\mathbb{L}_2 w = - \varepsilon \frac{\partial}{\partial y} \{ (\alpha y + \beta) f \} + \varepsilon \left[ f(y) + y f'(y) + f''(y) \right] \phi(u),
\]

from which, we can conclude
\[
\frac{\partial f}{\partial \tau} = -\varepsilon \frac{\partial}{\partial y} \{ (\alpha y + \beta) f \} + \varepsilon \frac{\partial}{\partial y} \{ y f(y) \} + \varepsilon \frac{\partial^2 f}{\partial y^2},
\]
in the original variables,
\[
\frac{\partial f}{\partial t} = -\frac{\partial}{\partial x} \left\{ \left( ax + b - \frac{k}{\gamma} x \right) f(x) \right\} + \frac{k_B T}{\gamma} \frac{\partial^2 f}{\partial x^2}.
\]
S4. WKB Theory

In this section we make some of the details of the WKB analysis more explicit. Most of this is taken from [23], but other resources can be found in [3, 4, 9, 13]. Specifically, we illustrate the significance of the principle eigenvalue. We first write the Fokker-Planck equation as the gradient of a flux

\[ \frac{\partial p}{\partial t} = -\nabla \cdot \{B(z)p\} + \frac{\varepsilon}{2} \nabla^2 p := -\nabla J(z, t) \quad (S6) \]

Let \( \tau \) denote the random variable describing the time for a trajectory to escape the basin of attraction of some equilibrium \( S \), denoted \( \Omega \). We can then define the survival probability \( S(t) \) that the trajectory has not escaped from \( \Omega \) yet by time \( t \). In other words,

\[ S(t) := \mathbb{P}[t > \tau] = \int_\Omega p(z, t) \, dz, \quad (S7) \]

from which we can compute the probability density \( f(t) \) using (S7) and the divergence theorem to yield

\[ f(t) = -\frac{dS}{dt} = -\int_\Omega \frac{\partial p}{\partial t} \, dz \quad (S8) \]

Define the operator and its adjoint

\[ \mathbb{L}_\varepsilon p := -\frac{\varepsilon}{2} \nabla^2 p - B \nabla p, \quad \mathbb{L}_\varepsilon^* p = -\frac{\varepsilon}{2} \nabla^2 p - \nabla \cdot \{Bp\}, \]

We now consider imposing absorbing (Dirichlet) boundary conditions on \( \partial \Omega \), that is

\[ p(z, t)|_{z \in \partial \Omega} = 0. \]

It can be proved that the eigenvalues of both \( \mathbb{L}_\varepsilon \) and its adjoint have a principle (smallest) eigenvalue that is real and exponentially small (that is, \( \lambda_0^\varepsilon \sim e^{-c/\varepsilon} \)) and also that the other eigenvalues satisfy \( 0 < \lambda_1^\varepsilon < \text{Re}\{\lambda_2^\varepsilon\} < \cdots \) with corresponding eigenvectors \( \phi_j^\varepsilon \).

Consider the eigenfunction expansion

\[ p_\varepsilon(z, t) = \sum_j c_j e^{-\lambda_j^\varepsilon t} \phi_j^\varepsilon(z). \]

For large \( t \), the principle eigenvalue dominates, meaning that

\[ p_\varepsilon(z, t) \sim c_0 e^{-\lambda_0^\varepsilon t} \phi_0(z). \quad (S9) \]

Thus, if we compute the mean first passage time, we see, using (S8) and (S9)

\[ \mathbb{E}[\tau] := \int_0^\infty tf(t) \, dt \sim \frac{1}{\lambda_0^\varepsilon}. \]

However, we can also consider integrating (S6) over the region with (S9) to see that

\[ \lambda_0^\varepsilon \int_\Omega \phi_0^\varepsilon(z) \, dz = \int_\Omega \nabla J_0^\varepsilon(z, t) \, dz, \]
which after using the divergence theorem, we get

$$\lambda_0 = \frac{\int_{\partial \Omega} J_0^0(z,t) \cdot n \, dz}{\int_{\Omega} \phi_0^0(z) \, dz}.$$  

In other words, the rate of escape is effectively the flux across the boundary and determined exclusively by the principle eigenfunction $\phi_0^0$. However, we recall that $\lambda_0^\varepsilon \sim e^{-c/\varepsilon}$ for some $c$. Thus, in the weak noise limit $\varepsilon \to 0$, $\lambda_0^\varepsilon \to 0$. Thus, rather than solve the principle eigenfunction equation $L_\varepsilon^* \phi_\varepsilon^0 = \lambda_\varepsilon^0 \phi_\varepsilon^0$, we can actually solve

$$L_\varepsilon^* \phi_\varepsilon^0 = 0,$$

that is, we need to find the \textit{quasistationary distribution} of the Fokker-Planck equation and make corrections to the boundary since solving for the eigenfunction using the WKB ansatz technique does not produce the appropriate absorbing boundary condition.
S5. Geometric Minimum Action Method

This section briefly describes the numerical scheme used to compute the most probable exit path: the geometric minimum action method. This technique was proposed in [13], which describes many more details and proofs related to the technique.

The motivation for this method comes from the integral (action) interpretation of the quasipotential described in (17). Specifically, we know that the quasistationary density is proportional to $e^{-\Phi(z)/\varepsilon}$, where, using (17), we want to consider the minimization of the action

$$S_T[z] := \frac{1}{2} \int_0^T \|\dot{z}(t) - B(z)\|^2 \, dt.$$  \hspace{1cm} (S10)

Note that this is an infinite dimensional minimization, as it’s also the minimum over all $T$ and paths $z$. First note the inequality

$$\|\dot{z}\|^2 + \|B(z)\|^2 \geq 2\|\dot{z}\| \|B\|$$  \hspace{1cm} (S11)

which implies that

$$\min_{z,T} S_T[z] \geq \min_{z,T} \hat{S}_T[z], \quad \text{where } \hat{S}_T[z] := \int_0^T \|\dot{z}\| \|B\| - \dot{z} \cdot B(z) \, dt.$$  \hspace{1cm} (S12)

In [13], two observations are made: $t$ can be re-parameterized such that the inequality (S11) becomes an equality without changing the actual path but ensuring that $\|\dot{z}\| = \|B(z)\|$ along the path. The other observation to make is that $T$ can be parameterized out of the line integral, as this will only change the value of $\hat{S}_T$, not whether it is minimized. Thus, we can just set $T = 1$ to find the actual path. One convenient way to parameterize is $\|\dot{z}\| = \text{constant}$, which is equivalent to thinking of $t$ as a normalized arc-length through our path. With these reductions, we now have the following problem:

$$\min_{\substack{z(s) \in \mathcal{S}\, z(0) = S, \, z(1) = H}} \hat{S}_1[z], \quad \text{subject to } \|\dot{z}(s)\| = \text{constant.}$$  \hspace{1cm} (S12)

Importantly, in [13], it is proved that the quasipotential is independent of whether (S10) or (S12) is used. That is, essentially,

$$\Phi(z) = \inf_{y: [0,T] \rightarrow \Omega} S_T[y] = \inf_{z: [0,1] \rightarrow \Omega} \hat{S}_1[z],$$

where again $z^*$ is one of the equilibria of the deterministic flow. Ultimately, we just need to find the minimizer of (S12), which must satisfy Euler-Lagrange equation $\frac{\partial \hat{S}_1}{\partial z} = 0$. Computing this functional derivative, we find

$$\lambda \frac{\partial \hat{S}_1}{\partial z} = -\lambda^2 \ddot{z} + \lambda (\nabla B - \nabla b^T) \dot{z} + (\nabla B)^T B - \lambda \dot{z},$$

where $\lambda := \|B\|/\|\dot{z}\|$ and $\nabla B$ is the Jacobian tensor of the system. Thus, to find when this functional derivative is zero, a preconditioned steepest-descent method in the direction of $-\lambda \partial \hat{S}_1 / \partial z$ is proposed.
Roughly, an initial path of grid points is formed, evolved via the steepest descent step, and then reparameterized such that the grid points are equal arc-length to ensure to the constraint is satisfied. In other words, take a path \( z^0(0) = z_1, z^0(1) = z_2 \) (where \( z_1, z_2 \) are typically equilibria of the deterministic system) and discretized into \( N + 1 \) equidistant points between the two, denoted \( z^0_i = z^0(i/N) \). Until convergence, we then repeat the process: given some discretized curve \( \{ z^k_i \}_{i=0,...,N} \), set \( \lambda^k_0 = \lambda^k_N = 0 \) and compute

\[
\dot{z}^k_i := \frac{(z^k_{i+1} - z^k_{i-1})}{2/N},
\]

as well as \( \lambda^k_i = (\lambda^k_{i+1} - \lambda^k_{i-1})/(2/N) \) for \( i = 1, \ldots, N - 1 \). Then, solve the tridiagonal system for \( \tilde{z}_i \) (using the Thomas algorithm)

\[
\frac{\tilde{z} - z_i^k}{\tau} = \left( \lambda_i^k \right)^2 \frac{\tilde{z}_{i+1} - 2\tilde{z}_i + \tilde{z}_{i-1}}{1/N^2} - \lambda_i^k \left[ \nabla B (z_i^k) - \nabla B^T (z_i^k) \right] \dot{z}_i^k - \nabla B^T (z_i^k) B (z_i^k) + \lambda_i^k \dot{\lambda}_i^k z_i^k
\]

with the boundary conditions fixing the endpoints \( \tilde{z}_0 = z_0^*, \tilde{z}_N = z_1^* \), where typically correspond to equilibria of the deterministic flow. Next, interpolate a set of equidistant \( N + 1 \) points across the interpolation of \( \tilde{x} \) and repeat. Along this path, the momentum \( p \) can be recovered using

\[
p_i = \lambda_i \dot{z}_i - B(z_i),
\]

the true time can be recovered from the choice of parameterization, by setting \( t_0 = 0 \) and

\[
t_i = \frac{1}{2\lambda_i^0} + \frac{1}{\lambda_i^1} + \cdots + \frac{1}{\lambda_i^{N-1}} + \frac{1}{2\lambda_i^N}.
\]

The action can be evaluated independent of the parameterization:

\[
\hat{S} = \frac{1}{N} \left( \frac{3}{2} \tilde{z}_1 \cdot p_1 + \sum_{i=2}^{N-2} \tilde{z}_i \cdot p_i + \frac{3}{2} \tilde{z}_{N-1} \cdot p_{N-1} \right).
\]

If other quantities, such as the prefactor are required, the shooting transport equations can be used along this geometric minimum path by the appropriate parameterization of \( t \) determined by (S13).