ADP release can explain spatially-dependent kinesin binding times

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Abstract

The self-organization of cells relies on the profound complexity of protein-protein interactions. Challenges in directly observing these events have hindered progress toward understanding their diverse behaviors. One notable example is the interaction between molecular motors and cytoskeletal systems that combine to perform a variety of cellular functions. In this work, we leverage theory and experiments to identify and quantify the rate-limiting mechanism of the initial association between a cargo-bound kinesin motor and a microtubule track. Recent advances in optical tweezers provide binding times for several lengths of kinesin motors trapped at varying distances from a microtubule, empowering the investigation of competing models. We first explore a diffusion-limited model of binding. Through Brownian dynamics simulations and simulation-based inference, we find this simple diffusion model fails to explain the experimental binding times, but an extended model that accounts for the ADP state of the molecular motor agrees closely with the data, even under the scrutiny of penalizing for additional model complexity. We provide quantification of both kinetic rates and biophysical parameters underlying the proposed binding process. Our model suggests that most but not every motor binding event is limited by their ADP state. Lastly, we predict how these association rates can be modulated in distinct ways through variation of environmental concentrations and spatial distances.

Significance Statement

Cytoskeletal-motor assemblies self-organize to achieve cellular functions ranging from delivering intracellular cargoes to generating forces in mitosis. Advancements in single-molecule experiments have revealed immense detail about motor detachment and stepping, but relatively little regarding the attachment process. With newly available spatially parameterized motor binding times from an optical trap, the evaluation of mechanistic models for binding becomes possible. We find that a model limited by both diffusive search and ADP-release best explains the data. The coupled chemo-mechanical nature of this interaction is more malleable than either separately, possibly explaining the rich diversity and regulation observed in cells. More broadly, our study provides a timely vignette on leveraging computations with experiments to understand how geometry and other complexities shape protein-protein interactions.

Introduction

Life depends on an immensely diverse and complex array of protein-protein interactions [1]. These interactions are richly regulated in both space and time (e.g., via post-translational modifications, fluctuating concentrations [2]) to modulate affinities, promiscuities, and sensitivities [3]. Understanding how these interactions are parameterized by both chemical and physical factors is broadly limited due to challenges in observing interaction events directly [4]. While predicting interactions from molecular structures (e.g., from molecular dynamics simulations) is an invaluable approach, these investigations still suffer from the same observational limitation in their validation [5].

One variety of such interactions of diffuse importance across cellular function are those between molecular motors and cytoskeletal filaments. Cytoskeletal motors, specifically kinesin-microtubule assemblies, self-organize to perform a zoo of cellular behaviors, including the delivery of cargoes in intracellular transport [6], generation of forces to guide genetic material in mitosis [7][8], and guiding of axonal growth [9]. Each of these wildly different behaviors is fundamentally achieved through molecular motors binding, stepping, and unbinding from cytoskeletal filaments [10]. Over the last several decades, advancements in single-molecule experiments have revealed extensive details about the latter two components [11][13]. Stepping and unbinding are, in some sense, downstream of binding, suggesting clear merit in understanding the details underlying the process.

Pursuits toward understanding motor-cytoskeleton binding have been clouded by complications in disentangling the measurements from convolving factors. That is, one must specify exactly the notion of binding that is being measured. To do so, consider the full process of self-assembly. Initially, a freely diffusing motor associates with a cargo, the motor-cargo complex then diffuses to close proximity to a cytoskeletal filament, and then the first motor binds to this cytoskeletal filament. After this initial binding, other motors associated with the cargo attach to the filament and go through cycles of reattachment. Due to the challenges in disentangling these steps, there is...
enormous variety in the reported ranges for binding rates. While landing rate assays provide direct measurements of motor-cargo association rates, these do not inform motor-cytoskeletal rates. With the exception of [15], very little data of direct measurements of motor-microtubule binding events exists, but this study corresponds to the reattachment of a secondary motor that is kept in close proximity to the filament by another. Effective binding on the timescale of seconds to tenths of a second have been reported from indirect measurements and utilized heavily in other modeling works to understand collective motor behavior. However, these effective rates neglect geometric factors (such as organization on the cargo) that are known to crucially dictate the binding rate. A mechanistic, biophysical model of the binding process is therefore necessary to reconcile the various experimental observations and modeling efforts.

Figure 1: Experimental setup. An optical trap (pink) controls the average z-position of a polystyrene microbead cargo. When the cargo-motor ensemble binds to the microtubule and begins to walk on it, a position-sensitive diode (PSD) senses the displacement of the bead. As the motor walks farther from the center of the trap, the force on the cargo (and consequently the motor) grows, eventually leading to unbinding from the microtubule and resetting of the setup. The PSD measurements provide the timing between unbinding to rebinding, the binding process modeled throughout the remainder of the work.

Results

Diffusion-limited binding does not capture the qualitative behavior of experimental data.

To investigate the biophysical mechanisms of the first association between a cargo-bound motor and a microtubule, we compare binding time data of three kinesin lengths (33, 45, and 60 nm) attached to a polystyrene bead that is laser-trapped at several distances away (0, 20, 40, and 60 nm) from a microtubule. Concentrations of motors in solution are diluted such that at most one motor is on each bead. Throughout the remainder of the work, we consider the binding time to be that between the unbinding reset event and the next time of detectable direction of the bead, as schematically shown in Fig. 1. See Materials and Methods for further details on the
Figure 2: Schematic of models. Left: State diagram of the simple diffusion model of kinesin-microtubule binding. Through random motion of the cargo and motor head, binding time is determined by the stochastic search process of the motor reacting when in close proximity to the microtubule. Right: Model of binding process that considers ADP release. We consider a cargo-motor ensemble that is in State 1, unbound from the microtubule and bound to ADP on both motor heads. From here, the motor can release ADP from one of its heads, transitioning to State 2, or bind weakly to the microtubule in State 3. To bind strongly to the microtubule and transition to State 4, the motor must meet two requirements: ADP is released from one of its motor heads and it must be within a binding distance to the microtubule. We consider two types of ADP release: a non-tubulin stimulated rate ($k_{ADP,off}$), and a faster tubulin-stimulated rate ($k_{ADP,Fast,off}$).
mean time to bind for the three motor lengths and various distances. There appears to be reasonable qualitative agreement with the experiments, where increasing distances increases binding time. The corresponding parameter fits can be found in Table 1. While the diffusivity of the motor head is challenging to quantify [30], our fitted value on the order of 1000-10000 nm$^2$/s is within ranges considered for kinesin elsewhere [31]. Upon further scrutiny, the mean binding times shown in Fig. 3ABC, especially at close distance display a distinct qualitative disagreement between the diffusion model and experimental measurements. In the diffusion model, as the cargo is trapped closer, the motor is effectively instantly able to bind. However, experimental values show a plateau of times around 1 second, even for close distances. This plateau points to the binding process being a multistep process.

![Figure 3: Model fits and cross-validation. ADP-release model captures qualitative behavior in experimental data, while the simple diffusion model cannot. In vitro optical trap experiments were used to measure mean binding times (black) for three motor lengths: 33 nm A, 45 nm B, and 60 nm C. The horizontal axis shows average distances between the cargo and microtubule (MT), which were varied for each experiment. Two binding models (simple diffusion only in blue and ADP-release in red) were simulated and fitted to the experimental data. $n = 100$ for simulated data varied for experimental data. Data are presented as mean ± SEM. D: Cross-validation was performed to determine the predictive power of each model. For three rounds, data was trained on two motor lengths, and tested on the third.](image_url)

A chemico-mechanical ADP-release model of binding better explains observed times.

With the observation that a simple diffusion model does not produce a delay in binding at close distances seen in experimental measurements, we sought a model that may explain this phenomenon. For a possible explana-
tion, we turn to the rich mechanochemistry of the kinesin motor. It is known that the nucleotide state of each motor head crucially determines its strong or weak affinity to the microtubule [32–34] and through cycles of this nucleotide state (ATP, ADP, released), processive stepping is achieved [35]. We posit that this nucleotide-based regulation of "binding" extends beyond that of processive stepping, and even the preliminary attachment between the cargo-motor ensemble and the microtubule. That is, we posit that the experimentally observed binding times correspond to a strong binding event, and therefore the underlying nucleotide state of the motor heads, plays a significant role in arriving at this state.

To investigate whether a model including nucleotide state may better explain the experimentally observed times, we extend the model to account for 4 possible states, as shown in [Fig. 2]. In this model, the motor-cargo ensemble begins in State 1 with both motor heads in an ADP state, undergoing the same random motion as the diffusion model. From here, the ensemble can enter one of two states: State 2, where ADP is released from either motor head, or State 3, when the ensemble diffuses close to the microtubule and weakly binds to it. From either of these states, the ensemble can then strongly bind to the microtubule either through diffusion (from State 2), or the ADP molecule is released (from State 3). We consider two types of ADP release, a fast tubulin-stimulated release and a slow non-tubulin-stimulated release [36–41]. We consider ADP release as a requirement for strong binding based on the neck-linker model for stepping where an ADP-bound head has a low affinity for the microtubule, then this trailing head moves forward along the microtubule bound to ADP, and when it steps down onto the microtubule, ADP is released [42]. Importantly, our description is coarse-grained to not track the heads separately, but we consider the ADP release to describe either motor head. The assumption that the ensemble begins in State 1 has two parts: we assume that if the motor detaches in an ATP-bound state, this phosphate release is fast [35], but then the corresponding ADP release is slow without tubulin [39].

Using the same simulation-based inference approaches for the diffusion model, we fit the observed binding times for all 3 motor lengths and distances simultaneously for the extended ADP-diffusion model, with 7 unknown parameters, 2 from the diffusion model, 4 reaction rates, and 1 corresponding to the strength of attachment in the weak binding state. The result of the fits can be seen in Fig. 3ABC. The overall fit is discernibly better for the 33 nm and 45 nm motor lengths, and arguably worse for 60 nm at long distances, but notably, the model now captures the qualitative feature of a plateau of times at short distances. While only the mean binding times were used to fit, [Figs. S.2 and S.3] show close agreement in full distributions of binding times as well.

Beyond the qualitative improvement, the inherent danger in quantitatively assessing whether the ADP-release model better explains the data comes from the increased model complexity [43]. Intuitively, a model with more parameters has more flexibility to produce a better fit, and careful attention must be paid to model selection. In lieu of commonly-used information-theoretic techniques (AIC, BIC), even for simulation-based inference [44], we instead leverage the structure of our experimental observations to compare models based on their ability to explain unseen experimental circumstances. We perform a cross-validation procedure where we fit both the diffusion and ADP-diffusion models to the binding times for 2 of the 3 motor lengths, withholding one for testing on the trained models. In each validation test of withholding a motor length, the more complex model generalized better, shown in Fig. 3D. From this, we conclude that the ADP-diffusion model better explains the observed binding times, even under cross-validation-based scrutiny [45].

Kinetic and biophysical parameters of the ADP-binding model can be estimated.

Beyond the qualitative lesson of identifying the ADP-diffusion model as explaining the data, our fitting procedure provides rich quantitative insight into the underlying processes by estimating underlying parameters, shown in Table 1 and Fig. 4. Some kinetic rates have been previously measured, and serve as support for the model, whereas others are, as far as we know, unmeasured. The values reported in Table 1 are point estimates from a simulation-based inference optimization procedure [27]. The values that have been previously measured agree within an order of magnitude. As validation of this optimization procedure for point estimates, we also performed a separate simulation-based inference technique, sequential approximate Bayesian computation (sABC) [28] to obtain samples of an (approximate) posterior distribution shown in Fig. 4. The reason for this method was two-fold: for one, the agreement between the point estimates arising from the two procedures validates the approximations involved in the techniques, and the latter sABC approach produces valuable uncertainty quantification that we were unable to employ but may very well be possible using the techniques of [27]. Further details on these procedures can be found in the Materials and Methods. Somewhat surprisingly, all parameters of the model seem to be identifiable, as shown in the relatively tightly-shaped posterior distributions.
Table 1: Fitted Parameters. $D_m$ is the diffusion constant of the tethered motor head. $\kappa_w$ is the stiffness constant of the weak bond between the motor and the microtubule when the motor is weakly bound to it. Parameters are fitted using a Bayesian optimization algorithm [27]. Some parameters were not found in previous literature.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Simple Diffusion Model</th>
<th>Diffusion w/ ADP State Model</th>
<th>Previous Literature</th>
</tr>
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<tbody>
<tr>
<td>$k_{\text{off}}^{\text{ADP}}$ (s$^{-1}$)</td>
<td>0</td>
<td>0.008</td>
<td>0.008-0.1 [36, 37, 40, 41]</td>
</tr>
<tr>
<td>$k_{\text{off}}^\text{fast}$ (s$^{-1}$)</td>
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<td>2.12</td>
<td>1.14-300 [37, 41]</td>
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<td>425 [41]</td>
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<tr>
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<td>70.652</td>
<td></td>
</tr>
<tr>
<td>$k_{\text{off}}^{\text{MT}}$ (s$^{-1}$)</td>
<td>0</td>
<td>0.2</td>
<td>0.31 [46]</td>
</tr>
<tr>
<td>$D_m$ (nm$^2$ s$^{-1}$)</td>
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<td>1994</td>
<td></td>
</tr>
<tr>
<td>$\kappa_w$ (pN nm$^{-1}$)</td>
<td>0</td>
<td>0.002</td>
<td></td>
</tr>
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</table>

Figure 4: Estimates of microscopic parameters from fitting the ADP-diffusion model. Joint (Approximate) Posterior Distribution of ADP release Model Parameters. Black curves in the marginal densities from approximate Bayesian Computation (ABC) are the posterior distributions, and the grey curves are the priors. A kernel density estimator [47] was applied to discrete samples to form posterior estimate curves.

Most motors strongly bind via tubulin-stimulated ADP release.

These quantitative estimates of the underlying microscopic rates also provide conceptual lessons about motor binding. Referring to the model schematic in Fig. 2, motors are able to achieve the strong binding state either through an intermediate weak binding state (State 3) after which ADP release occurs, or directly from a diffusing state (State 2). Although we do not observe these transitions directly, their relative proportions can be deduced from the data and can be seen in Fig. 5 for all motor lengths and distances. Fig. 5A shows that for all motor lengths, the frequency of the diffusion-based transition to strong binding (State 4) from diffusion (State 2) is more frequent for shorter distances. Moreover, the extent of decrease in these events is most extreme for shorter motors. These observations make sense in light of this being the diffusion-based search step. The more challenging of a search (longer distances, or shorter motor), leads to less frequent encounters. This then leaves...
Figure 5: Cataloging typical binding events. Motors typically bind via tubulin-stimulated ADP release. Portion of transitions from States 2 (panel A) and 3 (panel B) to 4 are plotted for each motor length with respect to mean distance between the microtubule and the cargo. C: Mean percent of transitions that occur from each state. The mean of all events from all experimental conditions (motor length and mean spacing between cargo and microtubule) were taken. Parameters from Table 1 were used.

the remaining pathway from the weak binding state, the frequency of which can be seen in Fig. 5B. For short distances, the frequency remains relatively constant but then increases for long distances. The overall frequency is the highest for the shortest motor and lowest for the long. These transitions intuitively complement those of the diffusive search component. While we have focused on the parameterization of these relative frequencies, the most important note is likely their overall magnitude. In Fig. 5C, we show our predicted percent transitions, averaged over all the experimental conditions, in which most (over 80%) of initial binding interactions arise via a preliminary weak binding state and subsequent ADP release (States 3 to 4). While there may seem to be a paradox that the transition rate between States 2 to 4 is very low, which one might interpret as the ADP released state having low affinity, we emphasize this quantity is a byproduct of the competing rates between physical binding and ADP capture. That is, State 2 does not have lower affinity for the microtubule, but rather, simply occurs less often than ADP rebinding. A full report of the relative proportions of each transition can be found in Figs. S.5 and S.6.

Binding rates can be distinctly modified by physical and chemical factors.

The quantity and structure of experimental data have thus far allowed for significant progress in understanding binding from retrodictive reconciliation with a model. We conclude with predictions that emphasize the broader lessons and may serve as the basis of validation in future experiments. The exploration of typical binding events...
Figure 6: Predicted binding time changes from chemical and physical perturbations. A: An example of a chemical change, $k_{\text{ADP, Fast}}$, was varied in the simulation and resulting simulated binding times are plotted. Data are presented as mean ± SEM. B: Physical changes, such as changing the cargo size, can also be made to study the resulting binding times.

in the previous section points toward conceptualizing this process as a distinct mechanical diffusive search and a chemical step from the nucleotide state. Regulation and perturbations of each of these components should therefore be discernible. To explore these two scenarios, we predict how the binding times should be altered in two hypothetical experimental perturbations shown in Fig. 6. In the first, panel A shows how if one could modulate the tubulin-stimulated ADP-release rate, the effective spatially-dependent binding rate shifts up or downward for all distances. In contrast, there are numerous physical experiments that could plausibly alter the physical properties of the system. In Fig. 6B, we show the predicted effect of changing the cargo size, which would consequently modify the overall diffusivity of the ensemble and the random search time. For short distances that are not limited by this diffusive search, the difference is negligible, but for long distances, the effect becomes magnified. Predictions for other motor lengths can be found in Fig. S.7. While we do not currently have the technological capability to validate these experimental predictions, we hope they will be the basis of future validation or invalidation of our proposed binding model.

Discussion

Conclusion

Altogether, our results point toward a model of the initial binding between a cargo-bound kinesin and a microtubule track being more complex than just diffusion-limited search of the motor head presumed elsewhere[10, 31]. Motivated by the known mechano-chemistry of motor stepping, where the nucleotide state of each motor head dictates microtubule affinity [36, 39, 48], we posit that the observed binding times correspond to a nucleotide-state-limited strong binding event. To validate this hypothesis, we considered a coarse-grained computational model that incorporates both diffusive search and the ADP state of motor heads, and through simulation-based inference and model selection, ultimately found compelling agreement with the experimental measurements both in the observed binding times and in agreement with estimated microscopic parameters measured elsewhere [37–41]. Even with only observed binding times, our model predicts that the “typical” binding event is modulated distinctly by both environmental and physical factors. From a design perspective, these orthogonal modulations allow for more fine-grained control and malleability than either separately.

Motor binding times have been estimated and measured many times prior [14–17], but these studies cannot easily disentangle the physical configuration the binding arises from, whether it be from a landing experiment, DNA origami, or motors re-attaching while another is already bound. The absence of this consideration jux-
tapes the increasing body of evidence that spatial organization plays a vital role in motor binding [23–26]. By distilling both the experimental assay and corresponding model to the minimal ingredients of a single motor attachment, we can clarify this process with unprecedented precision and generalizability. That is, while we have shown that our model successfully recapitulates the experimental data from our optical trap assay, we have moreover provided quantitative details about the underlying process that can be used to calculate binding times in other configurations. That is, one could imagine taking our fitted model and adapting it to DNA-origami cargo to reconcile the observed binding rates of [15, 17]. Discrepancies between the predictions and observations may occur, but these provide crucial details about the underlying chemistry and physics that we advocate warrant further investigation.

Limitations and Assumptions

We have not, and likely cannot ever fully rule out other conceptual models and confounding factors of binding time. The key qualitative feature we sought to replicate was the binding delay on the order of 1 second in close-proximity cargoes. Our ADP release model successfully recapitulates this, and we provide testable hypotheses that can be used to invalidate the model in Fig. 6. We considered several other possible factors that may explain or contribute to this delay. The most pressing possibility is whether this delay arises as an artifact of failure to detect optical trap displacements faster than this. Assuming an unloaded kinesin velocity of around 500 nm/s, a 15 nm bead displacement for detection corresponds to fractions of a second, and does not explain the observed delay on the order of a second. In our model, we assumed the motor was configured downward initially, but this is likely not the case. However, based on the estimates of a cargo taking 0.2 seconds to complete a full rotation at this viscosity [26], we do not believe this can explain the delay. Furthermore, we are unsure what a realistic initial configuration of the motor may be, as a more detailed model of the cargo resetting in the optical trap likely requires consideration of hydrodynamic effects that couple rotation and motion and distant-dependent diffusivity [49]. One last possibility we note is the conceptual model where a motor begins in a "crumpled" state, and then unfolds with some delay to bind. Our model of the kinesin stalk is crude, and one could imagine other possibilities such as a worm-like chain model considered for the neck linker [50]. However, these models primarily differ when under load, rather than undergoing a diffusive search. Therefore, we leave the investigation of other polymer models for the stalk to future work. We note the coarse-grained approximation of the whole motor head as a single spatial point with a single nucleotide state. We also leave to future work more detailed models with two distinct motor heads, as we do not believe this would greatly change the qualitative lessons emerging from our work. Lastly, we identify that this binding model may be limited to kinesin, and perhaps even only some kinesins. Future investigation warrants investigating other motors binding details, e.g., dynein, through the procedure outlined in this work.

Context in Cytoskeletal-Motor Systems

The focus of this work is understanding the binding between a single cargo-bound kinesin and a microtubule track. This setup follows the spirit of a now long-established and successful line of investigation of cytoskeletal-motor assemblies by isolating fundamental building blocks. We attempt to situate our advances in the broader context of the wildly complex array of cytoskeletal-motor interactions and the feedback between them. We emphasize the chemo-mechanical nature of our binding model in the context of the enormous literature on how physical and chemical changes to microtubules affect motor behavior. For instance, geometry of the microtubule network dictates cargo-microtubule distances [51, 52], and motors themselves reorganize microtubules through forces [31]. A zoo of microtubule-associating-proteins (MAPs) and the tubulin code are known to interfere with motor function [53, 54], including in the recruitment of motors [55–57]. Our model may shed light on explaining the mechanisms by which these microtubule decorations modify motor binding, and serve as the basis for future investigation. Through the decomposition of how chemical and physical factors modulate binding, our study may be the basis for discerning the mechanism of MAPs regulation of motors. For instance, one could imagine C-terminal tails may serve as physical tethers or may alter nucleotide states, and such an investigation remains for the future. More broadly, this understanding serves as a key step toward understanding how cells regulate binding to direct cargo and perform even more cytoskeletal-motor functions such as coordinate mitosis [58]. Moreover, this understanding may help aid in the design of increasingly sophisticated synthetic systems [59], where spatial distances can be prescribed.
Broader Lessons for Probing Protein-Protein Interactions

The difficulty in directly observing protein-protein interactions makes their study challenging. Two main avenues of approaches have been historically successful, each at extremes of chosen level of detail. Molecular dynamics simulations are a gold standard for predicting interactions. We build on the immense insight they have illuminated on the interactions between microtubules and kinesin \[60, 61\] and otherwise would not have considered the ADP release in our model. However, these approaches built from microscopic components have immense difficulty in scaling up to more complex systems with multiple interacting components, such as between motor, cargo, and cytoskeletal filaments. At the other extreme, “spherical cow” models of diffusion-limited reactions \[62, 63\] have revealed many qualitative lessons of protein-protein interactions, but remain challenging to quantitatively link with data because even the inclusion of modest complexities like orientation constraints \[64\] make the analyses prohibitively complex.

Our work adds a timely vignette to other studies \[65, 66\] that illustrates the value of striking an intermediate level of complexity in understanding protein-protein interactions. This balance allows for the incorporation of microscopic details from more fine-grained studies, but remains vigilantly coarse-grained to directly connect with data. We highlight major components of the work that we believe will be of broader use in other probing of protein-protein interactions. For one, we leveraged measuring interactions in a variety of conditions. Equipped with only a single motor length or trapped distance, the diffusion model would have fit to a seemingly satisfactory degree. By probing a model’s ability to explain data across conditions, we were able to identify the ADP model. Furthermore, our work was made possible by recent advances in simulation-based inference \[67\]. While model fitting has historically been bogged down in the complications of the procedure, we now live in an age where it is plausible to perform inference on any model that can be thought of (and simulated), with rapidly improving techniques beyond those we utilized in this work \[68\]. Neither of these lessons is specific to the context of cytoskeletal-motor interactions, and therefore we hope serves as an outline for other pursuits in data-driven discovery of protein-protein interactions.

Material and Methods

Optical Trap Experiments

The optical trapping setup was assembled on an inverted Nikon TE200 microscope using a 980 nm, single mode, fiber-coupled diode laser (EM4 Inc). The laser power was set to achieve a trap stiffness, $k_t$, of $\sim 0.045 \text{ pN nm}^{-1}$ while using the polystyrene bead of 0.56 $\mu$m (streptavidin conjugated, Spherotech).

Single motor experiments were carried out in the motility buffer (80 mM Pipes pH 6.9, 50 mM CH3COOK, 4 mM MgSO4, 1 mM DTT, 1 mm EGTA, 10 $\mu$M taxol, 1 mg mL$^{-1}$ casein). In all the rebinding rate assays, single-motor kinesin-coated polystyrene beads were prepared just before the measurements. The motors DK-406-His/K-560-His/DK-746-His (Kinesin-1, aa 1-560/Drosophila Kinesin aa 1-406/ Drosophila Kinesin aa 1-746; His tag at c-term) were diluted to $\sim 20 \text{nM}$ before mixing with $\sim 1 \text{mM}$ of biotinylated penta-His- antibody conjugated streptavidin beads stored at 4°C. This ratio produced the bead binding fraction of 10-15% and was maintained to maximize the probability of finding single motor beads in the solution. The bead-motor incubation ($\sim 50 \mu$L volume) was carried out at room temperature for 10 minutes. At the end of incubation, the sample chamber with preassembled microtubules was washed with $\sim 50 \mu$L of warm filtered buffer just before injecting the incubated mixture. Experiments were carried out at room temperature in a motility buffer supplemented with 2 mM ATP and oxygen-scavenging system (0.25 mg mL$^{-1}$ glucose oxidase, 30 $\mu$g mL$^{-1}$ catalase, 4.5 mg mL$^{-1}$ glucose).

In general, small dust or debris in the solution gets pulled into the trap along with the bead. Trapped dust interferes with motor rebinding to microtubules. To prevent this interference, the large dust particles and aggregates of casein in the buffer were removed using a 100 nm centrifugal filter (Millipore). Another potential issue is the stage drift during measurement, and it was minimized with an automated drift correction system using an xyz piezo stage (PI) and custom software.

All kinesin proteins were purified using HIS-tag and MT affinity purification after expressing them in Rosetta bacterial cells as described earlier \[69\]. DK406 plasmid was procured from Addgene (plasmid ID #129764, generously supplied by William Hancock lab). DK746 was designed in the lab after modifying the full-length DK980, also procured from Addgene (plasmid ID: #129762, William Hancock lab), using restriction enzyme digestion.
Binding Detection

Bead displacements in the trap registered by a position-sensitive diode (PSD; First Sensor AG) were acquired at 3kHz using an analog-to-digital converter (ADC) card. The digitized PSD data was smoothed with a 40 point fast Fourier transform (FFT) filter and analyzed by custom Matlab code to score all the peaks greater than 15 nm and lasting more than 0.01 seconds (30 data points). The experimental method is fully described in [70].

Brownian Dynamics Simulation

The simulation consists of a motor that is bound to a cargo and a microtubule. The cargo is a three-dimensional sphere and is subject to translational and rotational diffusion. The motor’s condition is dependent on whether an ADP molecule is bound to it and whether it is bound to the microtubule. Whether the motor is weakly or strongly bound to the microtubule is dependent on whether it is bound to an ADP molecule. The transitions through these states (Fig. 2) are simulated using a Gillespie-style algorithm [71]. The motor is defined by its location of attachment to the cargo and its head location. Locations of the motor head and cargo center are calculated using the Euler-Maruyama method [72]. When the motor heads comes within binding reach of the microtubule, it has a constant rate of binding to it; otherwise, this rate is 0. The motor behaves as a spring, and when they are bound, they experience and exert force. When the motor is weakly bound to the microtubule, its off-rate depends on force. ADP molecules can also bind and unbind to the motor head at constant rates, but the ADP off-rate is dependent on whether the motor is weakly bound to the microtubule. The equations of motion for the cargo and motor are constructed by discretizing a set of stochastic ordinary differential equations derived from force balance.

ADP Release Model Description

This model is three-dimensional and mesoscale. A set of stochastic ordinary differential equations is used to describe the location of the cargo sphere and the motor that is attached to it. The motor transitions stochastically between discrete states (Fig. 2), and these transitions occur as Poisson processes. The force that the motor exerts on the cargo is modeled as a one-way spring:

\[
\vec{F}^m \left( \vec{a}, \vec{h} \right) = \begin{cases} 
-\kappa^m \left( |\vec{h} - \vec{a}|-L_m \right) \left( \frac{\vec{h} - \vec{a}}{|\vec{h} - \vec{a}|} \right) + \vec{F}^w & \text{if } |\vec{h} - \vec{a}| > L_m \\
0 & \text{if } |\vec{h} - \vec{a}| \leq L_m 
\end{cases},
\]

(1)

where \(\vec{a}\) and \(\vec{h}\) are the motor anchor and head locations, respectively, \(\kappa^m\) is the motor stiffness constant, \(L_m\) is the motor rest length,

\[
\vec{F}^w = \begin{cases} 
\kappa^w \left( \vec{a}_{MT} - \vec{h} \right) & \text{motor is weakly bound to the microtubule} \\
0 & \text{motor is unbound from the microtubule} 
\end{cases},
\]

(2)

\(\kappa^w\) is the weak spring between the motor head and the microtubule, and \(\vec{a}_{MT}\) is where the motor head is weakly bound on the microtubule. There is a torque that is exerted on the cargo:

\[
\vec{\tau}^m \left( \vec{a}, \vec{h}, \vec{c} \right) = \begin{cases} 
(\vec{a} - \vec{c}) \times \vec{F}^m \left( \vec{a}, \vec{h} \right) & \text{motor is weakly bound to the microtubule} \\
0 & \text{otherwise} 
\end{cases},
\]

(3)

where \(\vec{c}\) is the cargo center location. Thus, we have ordinary differential equations (modeled after the Langevin equation):

\[
\frac{d\vec{c}}{dt} = \frac{1}{6\pi\eta R} \vec{F}^m \left( \vec{a}(t), \vec{h}(t) \right) + \frac{1}{6\pi\eta R} \vec{F}^b(t),
\]

(4)

and

\[
\frac{d\vec{\theta}}{dt} = \frac{1}{8\pi\eta R^3} \vec{\tau}^m \left( \vec{a}(t), \vec{h}(t), \vec{c}(t) \right) + \frac{1}{8\pi\eta R^3} \vec{\tau}^b(t),
\]

(5)
where η is the water viscosity, $R$ is the cargo sphere radius, $θ$ is the cargo orientation, and $\vec{F}^b$ and $\vec{\tau}^b$ are the Brownian force and torque, respectively, which are random variables with mean 0 and variance $2k_BT\xi_c$, and $\xi_c$ is the drag coefficient of the cargo. According to the Euler-Maruyama method, a discrete formulation of Eqs. (4) and (5) will be:

$$\vec{c}(t_{n+1}) = \vec{c}(t_n) + \frac{1}{6\pi \eta R} \vec{F}^m_n \left( \vec{a}(t_n), \vec{h}(t_n) \right) \Delta t + \sqrt{2 \frac{k_BT}{6\pi \eta R}} \Delta t \vec{G}_c(t_n)$$

(6)

and

$$\vec{\theta}(t_{n+1}) = \vec{\theta}(t_n) + \frac{1}{8\pi \eta R^3} \vec{\tau}^m_n \left( \vec{a}(t_n), \vec{h}(t_n), \vec{c}(t_n) \right) \Delta t + \sqrt{2 \frac{k_BT}{8\pi \eta R^3}} \Delta t \vec{G}_\theta(t_n),$$

(7)

where $n$ is the current time step, and $\vec{G}_c$ and $\vec{G}_\theta$ are mutually uncorrelated vectors of independent and identically distributed (i.i.d.) Gaussian random variables with mean 0 and variance 1. The cargo cannot phase through the microtubule. Since we are simulating optical trap experiments, we add a force from the trap on the cargo:

$$\vec{c}(t_{n+1}) = \vec{c}(t_n) + \kappa_t (\vec{c}(1) - \vec{c}(t_n)) + \frac{1}{6\pi \eta R} \vec{F}^m_n \left( \vec{a}(t_n), \vec{h}(t_n) \right) \Delta t + \sqrt{2 \frac{k_BT}{6\pi \eta R}} \Delta t \vec{G}_c(t_n),$$

(8)

where $\kappa_t$ is the trap stiffness. We can now determine the motor anchor location by inputting the cargo axis of rotation $\vec{\theta}(t_{n+1}) - \vec{\theta}(t_n)$ and this axis’ length as the magnitude of rotation (in radians) into a rotation matrix $\vec{M}(t_n)$:

$$\vec{a}(t_{n+1}) = \vec{M}(t_n) (\vec{a}(t_n) - \vec{c}(t_n)) + \vec{c}(t_n) + (\vec{c}(t_{n+1}) - \vec{c}(t_n)).$$

(9)

Similarly to Eqs. (4) and (5) we discretize ordinary differential equations for the motor head position:

$$\vec{h}(t_{n+1}) = \vec{h}(t_n) + \frac{1}{\xi_m} \vec{F}^m_n \Delta t + \sqrt{2D_m \vec{G}_m},$$

(10)

where $\xi_m = k_BT/D_m$ is the motor drag coefficient, $D_m$ is the motor head diffusion constant, and $\vec{G}_m$ is the uncorrelated i.i.d. Gaussian random variable of mean 0 and variance 1. The motor head cannot phase through the microtubule and the cargo. Since in experiments, the microtubule lies on the coverslip surface, the motor head cannot diffuse under the microtubule.

Transitions between each motor state (Fig. 2) are modeled as Poisson processes, with rates as follows:

$$\lambda_{\text{off}}^{\text{ADP}} = \begin{cases} k_{\text{off}}^{\text{ADP}} & \text{motor is ADP-bound and unbound from the microtubule} \\ k_{\text{off}}^{\text{ADP, Fast}} & \text{motor is ADP-bound and weakly bound to the microtubule} \\ 0 & \text{motor is ADP-unbound} \end{cases}$$

(11)

$$\lambda_{\text{on}}^{\text{ADP}} = \begin{cases} k_{\text{on}}^{\text{ADP}} & \text{motor is ADP-unbound} \\ 0 & \text{motor is ADP-bound} \end{cases}$$

(12)

$$\lambda_{\text{on}}^{\text{MT}} = \begin{cases} k_{\text{off}}^{\text{MT}} & \text{motor is unbound from microtubule and within } d_{\text{MT}} \\ k_{\text{off}}^{\text{ADP, Fast}} & \text{motor is weakly bound to microtubule} \\ 0 & \text{motor is not within } d_{\text{MT}} \end{cases},$$

(13)

where $d_{\text{MT}}$ is the binding distance between the motor head and the microtubule, and

$$\lambda_{\text{off}}^{\text{MT}} = \begin{cases} k_{\text{off}}^{\text{MT}} \cdot \exp \left( F^w/F^d \right) & \text{motor is weakly bound to the microtubule} \\ 0 & \text{motor is MT-unbound} \end{cases},$$

(14)

where $F^d$ is the motor’s critical detachment force. Parameter values are listed in Tables 1 and 2.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa_m$</td>
<td>Motor stiffness constant (pN/nm)</td>
<td>0.3200</td>
</tr>
<tr>
<td>$\kappa_t$</td>
<td>Trap stiffness (pN/nm)</td>
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</tr>
<tr>
<td>$L_m$</td>
<td>Motor length (nm)</td>
<td>Varies</td>
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<tr>
<td>$\eta$</td>
<td>Viscosity of experimental fluid (pN·s/nm²)</td>
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<tr>
<td>$R$</td>
<td>Radius of experimental cargo bead (nm)</td>
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<tr>
<td>$k_BT$</td>
<td>Boltmann constant (pN·nm)</td>
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</tr>
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<td>$d_{MT}$</td>
<td>Binding range between MT and motor head (nm)</td>
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</tr>
<tr>
<td>$F_{d}$</td>
<td>Motor critical detachment force (pN)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2: Measured parameters. *Unmeasured estimate. †Measured estimate. The motor length includes the antibody that binds the motor to the cargo (about 10 nm).

Initial Conditions

The simulation begins with the cargo $d$ nm away from the microtubule, the latter of which does not move during the entire simulation (Fig. S.1a). The motor is anchored at the bottom of the cargo and is vertically straight, based on the fast reset back to the trap center. If there is not enough space ($d < L_m$), then the motor head and cargo are initialized to the top of the microtubule.

Numerical Simulation

The model is simulated forward in time. Time steps are either equal to $dt_{\text{max}}$, the maximum time step the system can undergo, or they are determined through the Gillespie-style algorithm if the next motor state-change event (i.e., bound or unbound to microtubule, ADP released or unreleased), also determined by the Gillespie-style algorithm, occurs before $t_n + dt_{\text{max}}$. An appropriate $dt_{\text{max}}$ (0.004) was chosen with a convergence test (Fig. S.8). To implement the Gillespie-style algorithm, exponential random variables from distributions with means set by each Poisson (Eqs. (11) to (14)) were generated at each time step. After the Gillespie-style algorithm determines the next event and when it occurs, the time step is used to determine the locations of the cargo center and the motor’s head and anchor (Eqs. (8) to (10)).

To mimic experimental practices, simulations are allowed to simulate 100 seconds. If the motor does not strongly bind to the microtubule during this time, the simulation starts over. This method is similar to the experiment, where the assay is run for 100 seconds screening for a binding event to occur before trapping a different cargo. The simulation is written in MATLAB, and takes approximately 0.1 seconds to simulate 1 second of the system. Example snapshots of the simulation are shown in Fig. S.1.

Model Fitting, Cross-Validation

The model is fit through two distinct approximate inference procedures, the reconciliation of which serves as a validation for the approximations. The first procedure is a Bayesian optimization procedure [27] to obtain a single point estimate for the parameter values. The loss function is the squared distance over the mean binding times (and therefore neglects the full distributional information) and the estimated mean binding time over $S = 1000$ simulations, and these estimates are used in Fig. 3 and Table 1.

To obtain uncertainty quantification seen in Fig. 4, we also employ a sequential Monte Carlo approximate Bayesian computation approach [28]. These techniques are far slower than the optimization procedure and require the specification of a prior distribution for each parameter, but provide some notion of uncertainty quantification, and were used to generate Fig. 4 with some data withheld. That is, because of the heavy computation expense, only the shortest motor at 0 nm average distance between the cargo and microtubule, the mid-length motor at 40 nm average distance, and the longest motor at 80 nm average distance were used in the fitting. The maximum a posteriori (MAP) estimates from this latter procedure closely agree those of the first procedure, supporting the validity of both. Furthermore, in Table S.1 we show the inference procedures successfully infer rates from synthetic data. Lognormal priors are chosen for all parameters, and hyperparameters are shown in Table 3. Initially, 100 simulations estimate the binding times in the model, and weights in the sequential Monte
Carlo algorithm are defined as \( w_i = \pi P w_{i-1} K_i \), where \( K_i \) is the perturbation kernel for the \( i \)th sequence, \( i > 1 \). We use a Gaussian distribution for \( K \). These new samples are then used to simulate more mean binding times, until 100 samples are generated that resulted in a relative error lower than 1.8. Eight more sequences follow in this same manner, each time the relative error threshold decreases by 0.2. A kernel density estimator was then applied to the resulting samples shown in Fig. 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{ADP} ) off</td>
<td>( 10^{-2} )</td>
<td>0.001</td>
</tr>
<tr>
<td>( k_{ADP} ) on</td>
<td>( 10^3 )</td>
<td>100</td>
</tr>
<tr>
<td>( k_{ADP, Fast} ) off</td>
<td>( 10^{0.3} )</td>
<td>1</td>
</tr>
<tr>
<td>( k_{MT} ) on</td>
<td>( 10^{1.7} )</td>
<td>100</td>
</tr>
<tr>
<td>( k_{MT} ) off</td>
<td>( 10^{-1} )</td>
<td>0.01</td>
</tr>
<tr>
<td>( D_m )</td>
<td>( 10^{3.3} )</td>
<td>100</td>
</tr>
<tr>
<td>( \kappa_w )</td>
<td>( 10^{-2.7} )</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Table 3:** Hyperparameters for priors used in estimated posterior densities, all taken to be lognormal distributions.

The cross-validation procedure in Fig. 3 was implemented by fitting the models using the aforementioned point estimate optimization scheme with data withheld, and then test error defined to be \( N^{-1} \sum_{i=1}^{N} (t_i - \hat{t}_i)/t_i \), a percentage error over the test scenarios. This procedure is validated in Fig. S.9 that shows cross-validation successfully identifying the correct model when tested against synthetic data.

**Software Availability**

MATLAB code to reproduce our results (compatible with version R2020a) is available at [https://github.com/trininguyen/MotorBinding/tree/master](https://github.com/trininguyen/MotorBinding/tree/master).

**Acknowledgements**

This work was supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1839285 and the National Institutes of Health under Grant R01 GM123068. Figure 2 includes an icon (lipid-hdl-1 icon) by Servier [https://smart.servier.com/](https://smart.servier.com/) which is licensed under CC-BY 3.0 Unported. This work utilized the infrastructure for high-performance and high-throughput computing, research data storage and analysis, and scientific software tool integration built, operated, and updated by the Research Cyberinfrastructure Center (RCIC) at the University of California, Irvine (UCI). The RCIC provides cluster-based systems, application software, and scalable storage to directly support the UCI research community. [https://rcic.uci.edu](https://rcic.uci.edu).

**References**


The kinesin-13 MCAK has an unconventional ATPase cycle adapted for microtubule depolymerization. The EMBO Journal (2011).


[38] W. Hancock and J. Howard. “Kinesin’s processivity results from mechanical and chemical coordination between the ATP hydrolysis cycles of the two motor domains”. PNAS (1999).


Figure S.1: Simulation Snapshots. The simulation starts as in (a), where the motor (red line) is anchored (blue dot) to the bottom of the cargo (green sphere). The microtubule (turquoise cylinder) is centered at (0,0), and the axes depict locations of other components with respect to the microtubule center, in nanometers. As the simulation continues, the cargo and the motor diffuses with respect to force laws (b). The simulation ends when the motor strongly binds to the microtubule (c). Time (seconds) at which each event occurs is shown above figures.
Figure S.2: Full distributions of binding times. Simulated data from ADP release model (red) is plotted over experimental data (blue). Overlap between distributions is shown in grey.
Figure S.3: Cumulative distributions of binding times. Simulated data from ADP release model (red) is plotted over experimental data (blue). Same information as Fig. S.2 but CDF instead of PDF.
Figure S.4: Influence of motor head diffusion on binding time. A parameter sweep of the diffusion constant was conducted. Data are presented as mean ± SEM.

Table S.1: Posterior Density Method Validation. Fake data was simulated using the ADP release model and the Simulated Values. A sequential Monte Carlo approximate Bayesian computational algorithm was performed on the fake data and recovered the simulated values (Estimated Value).
Figure S.5: Macroscopic rates of state transitions. Rates for each transition in the ADP+Diffusion model estimated with respect to average distance between the microtubule and the cargo for each motor length. Parameters from Table 1 were used.
Figure S.6: Proportion of Transitions. Two transitions are possible for each state, and portion of each transition are graphed. Parameters from Table 1 were used.
Figure S.7: Other Parameter Sweeps on Binding Times. The ADP release model. Data are presented as mean ± SEM.
Figure S.8: Time step convergence study. Maximum time step was varied and converged to a common binding time. 0.004 was the largest maximum time step that resulted in a binding time that is relatively similar to the results from smaller maximum time step. \( n = 1000 \).

(a) Synthetic data from ADP release model  
(b) Synthetic data from Simple Diffusion model

Figure S.9: Cross-Validation Test. Synthetic data was simulated using either the ADP release model (a) or the Simple Diffusion model (b), and both the ADP release and the Simple Diffusion models’ performance were evaluated using k-fold cross-validation.