Chapter 6

Kinesin recycling in stationary membrane tubes

Collections of motors dynamically organize to extract membrane tubes. The tubes dynamically grow but often pause or change direction as they traverse an underlying microtubule network. We find, in stalled membrane tubes in vitro, motor clusters begin to accumulate and reach the tip of a membrane tube at regular time intervals. The average times between cluster arrivals scale linearly with the time over which motors depart from the tip suggesting that motors are recycled towards the tip. Numerical simulations of the motor dynamics in the membrane tube and on the MTs show that the presence of cooperative binding between motors quantitatively accounts for the clustering observed experimentally. Cooperative binding along the length of the MT, cooperative unbinding at the tip and a nucleation point at a distance behind the tip define the recycling period. Based on comparison of the numerical results and experimental data we estimate a binding probability and concentration regime where the recycling phenomenon occurs. ¹

6.1 Processive motors in non-moving membrane tubes

Transportation within the cell is driven by mechanoenzymes, motor proteins. Motors not only deliver cargo, in the form of vesicles and other proteins, as they walk on cytoskeletal tracks, but they are also responsible for continuous reorganization of membrane compartments. Because of their essential cellular function, the physical properties of individual motor proteins have been heavily investigated. For example, the kinesin motor transports cargo to the plus ends of microtubules (MTs) as an ATPase,\(^3\) it can walk at speeds up to \(1\mu m/s,^{15}\) stalls at forces greater than \(5pN,^{9,11,14}\) and takes anywhere between \(50 - 100\) steps on a MT before it dissociates from the MT.\(^89\) However, there is more and more evidence that cooperation between multiple motors in cargo movement is critical for regulating cargo transport in cells.\(^30,90-92\) Though we know much about individual motor proteins, our understanding of how motors behave as collectives is still limited.

To study collective motor dynamics, we use a minimal model system where kinesin motors are specifically attached to giant unilamellar vesicles (GUVs). When the motor-coated GUVs encounter a surface decorated by MTs, in the presence of ATP, the motors extract membrane tubes.\(^49\) Because a single motor can only provide \(5pN\) of force\(^9,11,14\) and deformation of a vesicle to extract a membrane tube requires \(\approx 25pN,^{47,93}\) motors must work together to share the load. Motors have been shown to dynamically associate at the tips of growing membrane tubes so that collections of kinesins are readily available to pull the tube.\(^49,50\) These motor dynamics have been observed in growing membrane tubes. However, \textit{in vivo}, membrane tubes can be seen pausing and changing direction regularly (see movie of membrane tubes \textit{in vivo}\(^74\)). The dynamics of motor proteins in membrane tubes that are paused have not yet been investigated.

Here, we examine the dynamics of processive kinesin motors in stalled membrane tubes \textit{in vitro}. We find that motors repeatedly congregate en
route to the tip of the membrane tube at regular time intervals. Moreover, we find that the average time for clusters to form scales linearly with the time over which motors depart from the tip. We explain the clustering mechanism by cooperative binding: motors have a higher probability of binding to the MT nearby motors that are already bound, than to an unpopulated area of the MT. With a simple, 1-D lattice model, we are able to describe the motor behavior and further probe the dynamics with numerical simulations. Simulations that account for cooperative binding in concert with cooperative unbinding of motors at the tip of the membrane tube and a cluster nucleation point behind the tip where the membrane tube is held to the MT by a few motors, recover the linear relationship between average arrival time and tip decay time found in experiments. From simulations we estimate the probability of cooperative binding to be 0.24 and determine a critical number of motors on the tube, $25 < N < 120$, necessary for this phenomenon to occur in non-moving tubes.

6.2 Experimental results: kinesins cluster towards the tip at typical timescales

We use a minimal *in vitro* model system where kinesin motors are specifically attached to a fluorescently labeled lipid on Giant Unilamellar Vesicles (GUVs) to directly examine motor dynamics during membrane tube formation. We use kinesins (kinesin-1) because they are responsible for *in vivo* transport of vesicles and membrane material towards the plus end of MTs. *In vitro*, kinesins have been shown to collectively extract membrane tubes from a GUV as they walk on underlying MTs. The groups of kinesins walk towards the plus-end of the underlying MT with speeds of $370 \pm 43 \text{nm/s}$, comparable to MT gliding speeds for the kinesin construct we use. The motors accumulate at the tip of the growing membrane tube where their speeds are damped by the tube-pulling force. At some point, motors encounter the end of a MT (or a MT junction) and the tube can no longer be pulled forward, though the motors are still highly active.
Figure 6.1: **Kinesin dynamics in membrane tubes** a) Membrane tube network formed by kinesin motors. The image is a sum of a series of images tracing fluorescent kinesin dynamics in a membrane tube network. The star indicates the point at which the membrane tube is connected to the underlying microtubule (MT). bar= 5μm. b) Kymograph tracing the motor dynamics in the direction of the arrow of (a) in time. The arrows indicate examples of new kinesin motor clusters. c) Trace of the intensity profile in time at the tip and 1μm behind the tip, indicated by the arrows in (b). The two signals are generally anticorrelated so that when there is a high intensity at the tip the intensity behind the tip lowers. The region in the dashed circle shows two distinct anticorrelated parts of the intensity signals, indicated by the arrows.
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Figure 6.2: **Motor cluster timescale**

(a) Autocorrelation curve in time, averaged for all points along the membrane tube of fig. 6.1a and b. The correlation curve shows distinct peaks at \( \approx 11 \) s and 22 s. b) The peak around 11 s is confirmed by a peak in the power spectrum. c) Autocorrelation curve at the very tip of the membrane tube. The curve is fit with an exponential decay. The decay time of this fit tells the time, \( 12.6 \pm 0.5 \) s, it takes for clusters at the tip to dissipate. d) Plot of the decay (release) time of the motors at the tip of a tube vs. the typical cluster arrival time for different tubes. Different tubes have different characteristic times, but the times at which motor clusters form is linearly related to the release of motors from the tube where \( t_{\text{decay}} = (0.85 \pm 0.04)t_{\text{arrival}} \).
Fig. 6.1a shows the sum of a series of images of a tube network formed by kinesin motors. The fluorescence signal appears wherever there are motors bound to the MT. At and beyond the star in fig. 6.1a, the fluorescence rapidly decreases because the membrane tube is lifted from the surface and not attached to the MT. Though the tube is stationary, motors are still highly active in the tip region close to the MT. We trace the motor positions through time as they walk towards the tip of the membrane tube, along the tube in the direction of the dashed arrow. In the resulting kymograph (fig. 6.1b), the motors congregate to form clusters. The signal of motors at the tip is anticorrelated with the signal of motors behind the tip as shown by the intensity traces at the tip and 1μm behind the tip in fig. 6.1c. The region within the dashed oval shows two examples, indicated by the arrows, where the signal is anticorrelated. The anticorrelation suggests that the majority of the motors in the system collect into clusters in the same region so that, for example, when all the motors are bound to the MT near the tip, there are no motors available to bind behind the tip.

The distinct clusters of motors arrive at the stationary tip at regular time intervals. In the kymograph in fig. 6.1b, motors cluster approximately every 10 to 11s. We verify the times quantitatively by examining the time autocorrelation of the fluorescence signal from the motors (fig. 6.2a). The curve is an average of all correlation curves along the length of the membrane tube. The autocorrelation curve shows distinct peaks at 11s and 22s. This period of 11s is confirmed by examination of the power spectrum of the signal in fig. 6.2b. Also visible in the kymograph in fig. 6.1b, the motors appear to accumulate on average about 2μm behind the tip. In all stationary tubes we observe, we find that motor clusters accumulate and move towards the tip at regular time intervals. We compare this arrival time to the time at which motor clusters leave from the tip. The time it takes for a cluster to leave from the tip is determined by the decay time of the autocorrelation curve at the tip of the membrane tube. Fig. 6.2b shows an exponential fit to the autocorrelation curve from the tip region of the tube in fig. 6.1a. The exponential fit gives a decay time for the motors at the tip: 12.6 ± 0.5s. The times
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differ for different tubes but the motor decay times are linear with the times of arriving clusters (fig. 6.2c) where $t_{\text{decay}} = (0.85 \pm 0.04)t_{\text{arrival}}$. Because the time for motor arrival is linear with the time for motors to depart, we suggest that the motors are recycled towards the tip in paused membrane tubes. The recycling only arises in tubes that are no longer growing. The behavior may be present in growing tubes but so many motors accumulate at the tip that the fluorescent signal in the tip region is too high to be able to see the subtle motor dynamics we describe here. It should also be noted that diffusion alone cannot account for the time scale of this recycling pattern. Motors can diffuse in the membrane a distance of 2μm in less than 1s.\(^{53}\)

Figure 6.3: **Motor cluster dynamics** a) Intensity profile of a cluster of motors (following the dashed line in fig. 6.1b) moving towards the tip of a membrane tube: the fluorescence increase indicates that the cluster accumulates motors as it moves towards the tip. b) Cartoon showing the geometry of a membrane tube of length $L$ extending from a GUV. The tube is anchored to the MT a distance $X$ behind the tip.
In order to understand how the motors form clusters, we examine the motor density profile as motors move towards the tip. Each time a motor cluster reappears, the motor density starts small and increases as the motors move towards the tip of the membrane tube. The accumulation of motors is indicated by an increase in the fluorescence intensity profile of a building cluster (following the dashed line from fig. 6.1b) shown in fig. 6.3a. Though we cannot identify the exact location where the clusters begin to form, they always increase in number as the motors move towards the tip.

We postulate that a nucleation point is defined by the point at which a few motors close to the vesicle randomly anchor the membrane tube to the MT. This occurs at a distance $X$ behind the tip of the tube indicated by the star in fig. 6.1a and shown in the cartoon in fig. 6.3b. This geometry has been observed experimentally\textsuperscript{50} and is a shape that minimizes the energy of the GUV/membrane tube system by minimizing the curvature at the point where the tube meets the GUV.\textsuperscript{79,94} The location of a nucleation point can also be at the crossing of two underlying MTs, where membrane tubes are often found to bend and diverge, or formed at a point where the MT has a defect.

### 6.3 Model and Simulations: cooperative binding, unbinding and a nucleation point

The formation of clusters that arrive at regular time intervals can be explained in a physical picture in which motors diffusing in the membrane may randomly bind at a nucleation point a distance $X$ behind the tube (cartoon in fig. 6.3b). Once a few motors have bound to the MT and walk towards the tip, motors diffusing in the membrane tube have a high probability of binding to the MT next to motors already bound to the MT. This cooperative binding could arise from an increased proximity of the membrane tube to the MT lattice when a single motor links the tube to the MT making it easier for another motor nearby to bind. Another possible cause of cooperative binding could be mutual interaction...
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between motors.\textsuperscript{54,95} We further propose that motors at the tip of the membrane tube unbind cooperatively. As many motors accumulate in the tip region, individual motors that are unable to step forward will get frustrated and fall off initiating a cascade of motor detachment.\textsuperscript{96}

Figure 6.4: Simulations with motor cooperativity a) Motors bind randomly anywhere along the MT lattice with a probability $p_b$ and a distance $X$ behind the tip of the membrane tube with a probability $p_{b(X)}$. However, if a diffusing motor neighbors a motor that is already bound to the MT lattice, the diffusing motor will bind next to it on the MT with a probability $p_b^*$. On the MT lattice, motors may walk towards the tip of the MT with a probability $p_v$ or detach from the MT with a probability $p_u$ and at the very tip with a probability $p_u^*$.

We use Monte Carlo simulations to investigate whether or not a nucleation point, cooperative binding and cooperative unbinding at the tip account for the trends in our experimental data. We consider a MT directly beneath a membrane tube with $N$ diffusing motors. The high curvature of the membrane tube only allows $\approx 3$ protofilaments of the MT to be accessible to the motors in the membrane tube. We consider the simplest case and simulate the motor dynamics on a single protofilament and in a one-dimensional membrane tube. We consider a single motor to be a unit, neglecting the existence of different attachment and
detachment rates for both motor domains\textsuperscript{97} and all rates apply to the entire motor.

Motors diffusing in the 1-D membrane tube explore a length $l = \sqrt{4Dt}$, where $D$ is the diffusion constant, and $t$ is time. These diffusing motors do not feel each other and may occupy the same lattice site. Motors in the membrane tube may bind to the MT at a nucleation point a distance $X$ behind the tip of the MT, provided a lattice site is empty. Motors in the membrane may also bind to empty lattice sites on the MT next to already bound motors with a high probability. Once bound to the MT, a motor walks forward as long as the site in front of it is unoccupied.

The cartoon in fig. 6.4 shows the probabilities that govern motor behavior in the membrane tube and on the MT in the simulations. Motors freely diffusing in the membrane tube randomly bind to the MT lattice anywhere with a very small probability $p_b$, and at the nucleation point a distance $X$ behind the tip with a probability $p_b(X)$. If the diffusing motor encounters motors that occupy neighboring lattice sites on the MT, it binds to the MT with a probability $p_b^*$, where $p_b^* = \gamma p_b(X)$. Once motors are bound to the MT, they walk towards the tip at a constant velocity with a probability $p_v$. Motors unbind from the lattice with a probability $p_u$. At the tip of the tube, motors are initially less likely to fall off due to crowding effects,\textsuperscript{98,99} but as more motors accumulate individual motors will get frustrated and fall off initiating a cascade of motor detachment.\textsuperscript{96} Thus, we estimate the probability of unbinding, $p_u^* = \frac{p_u}{100}N$.

The values $D$, $V$, $p_v$, and $p_u$ used in the simulations are taken directly from experimentally measured values. $D = 1.2 \pm 0.2 \mu m^2/s$ for a lipid-motor complex freely diffusing in a membrane tube.\textsuperscript{53} The kinesin motor in these experiments walks with a probability $p_v = 1$ at speeds of $450 \pm 50 \text{ nm/s}$ which is $\approx 53 \pm 7 \text{ steps/s} \ (V)$\textsuperscript{100} Kinesins walk on MTs for an average of 100 steps\textsuperscript{13,14} ($p_u = 0.01$). We assume the probability of random binding anywhere along the MT lattice between $X$ and the tip to be very small $p_b = .001$.\textsuperscript{86} The small value is chosen since a motor is likely to diffuse in the membrane for a long time before “feeling” the MT below, because the majority of the lipid bilayer of the tube is not close to
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Figure 6.5: Simulations with cooperative binding and X a) Kymograph from a simulation where motors bind cooperatively and there is a nucleation point along the MT, X. \( N = 100, L = 10\mu m \) and \( X = 5\mu m \). Motor clusters appear approximately every 20s. b) Autocorrelation curve of the signal in (a) showing a distinct peak at \( \approx 20.8s \). c) Power spectrum of the signal with a peak at \( 20.4s \). d) Autocorrelation curve of the fluorescence signal at the tip of the membrane tube fit with an exponential decay that gives a cluster dissipation time of 17s. The cluster arrival times and decay time at the tip have similar values.
the MT. At $X$ the probability of binding is larger because the membrane tube is closer to the MT making the MT more accessible to motors in the membrane tube at this point. Thus, the probability of binding chosen here is $p_b(X) = .02$. As soon as motors feel clusters of motors on the MT below, they bind with an enhanced probability by a factor $\gamma = 12$. We reason the value of $\gamma$ by assuming a minimum cluster to be at least 2 motors and considering that there are at least 6 lattice sites around an individual motor on a MT that can be occupied by a neighboring motor. We use the above values for all the simulations.

Because the number of motors in a membrane tube, the length of a membrane tube and the point where the tube is anchored to the MT are different in each experiment, we also vary the values in the simulations to see how the system responds. Based on experimental conditions where vesicles have $\approx 120 \text{ motors/} \mu\text{m}^2$, we estimate the number of motors, $N$, on a membrane tube to be between 25 and 120. In our simulations, tubes that have fewer than 25 motors, often do not have motors at the tip of the tube implying that too few motors cannot support this tube system. Tubes with greater than 120 motors become very crowded at the tip and the dynamics of motor clusters can no longer be seen. Note that $N$ does not vary in an individual simulation because we assume the density of motors over the vesicle and tubes to be uniform. We consider membrane tubes that range from 5$\mu\text{m}$ to 10$\mu\text{m}$ in total length ($L$). (Simulations of tubes of length $> 10\mu\text{m}$ show the same quantitative results as tubes of 10$\mu\text{m}$). Distances behind the tip ($X$) range from 2$\mu\text{m}$ to 7$\mu\text{m}$.

Fig. 6.5a shows a kymograph from a simulation where $N = 100$, $L = 10\mu\text{m}$, and $X = 5\mu\text{m}$. The kymograph shows the formation of clusters arriving at the tip in intervals of $\approx 18s$. The autocorrelation (fig. 6.5b) and power spectrum (fig. 6.5c) of the signal confirm a cluster arrival time of $\approx 20s$ and motors decay from the tip over a time of $17s$ (fig. 6.5d). In contrast, in the absence of a nucleation point motor clustering requires a much higher probability of cooperative binding and clusters do not arrive at regular time intervals (fig. 6.6a). In this case $N = 40$ and $L = 10\mu\text{m}$. $N$ larger than 40 in simulations without $X$ lead to saturating conditions at the tip, so we show an example with fewer
Figure 6.6: **Simulations with cooperative binding but no X** a) Kymograph where motors bind cooperatively to the MT but where there is no defined nucleation point (no X). $N = 40$, $L = 10$, $p_b = 0.001$ and $p_b^* = 0.3$. Motors cluster, but there is less of a defined arrival period as in fig. 6.5a. b) Autocorrelation curve of the signal in (a) showing a less defined peak than in fig. 6.5b of $\approx 12.2s$. c) Power spectrum of the signal with a peak at $7.3s$. d) Autocorrelation curve of the signal at the tip of the tube with an exponential decay that gives a decay time of $11s$. The cluster arrival times and decay time at the tip are of the same order of magnitude though the peaks in the autocorrelation curve are not very large. e) Scatterplot of simulated data for different motor number ($L = 10 \mu m$) in the absence of a nucleation point, X. The different symbols represent different N. There is no linear increase in cluster arrival time with tip decay time as seen in the experimental data.
Figure 6.7: **Simulations without cooperative binding or $X$** a) Kymograph of motors walking along a MT below a membrane tube in the absence of both cooperative binding and $X$. $N = 40$, $L = 10$, $p_b = 0.001$, $p_{b}^{*} = 0$. In the absence of cooperative binding, motors do not cluster. b) The autocorrelation curve of the signal in (a) does not show any peaks in the correlation at longer time lags elucidating the absence of clusters. c) The power spectrum does not peak at any specific frequency as in figs. 6.5c and refsimdat2c. d) The exponential fit to the autocorrelation curve of the signal at the tip of the tube gives a time of $\approx 2s$. Because motors do not build into clusters and accumulate at the tip, the loss of motors at the tip is less significant.
motors here. The probability of cooperative binding is 300 (instead of 12) times greater than \( p_b \) because smaller values do not lead to cooperative binding in the absence of \( X \). Here, the highest peak appears to be at \( \approx 12.2 \) s (fig. 6.6b). The power spectrum suggests an average arrival time of 7.3 s (fig. 6.6c). The decay time, 11 s, is a similar value to the arrival time (fig. 6.6d). Fig. 6.6e shows the resulting scatterplot of average motor cluster arrival time vs. decay time at the tip for simulated data with different \( N \) in the absence of \( X \). There is no linear increase in cluster arrival time with tip decay time as seen in the experimental data. Moreover, if cooperative binding is absent, clusters do not form as can be seen in the kymograph of fig. 6.7a. Peaks in both the autocorrelation curve and the power spectrum are lost, in striking contrast to figs. 6.5b and 6.6b.

We have confirmed that a nucleation point is critical for the recycling phenomenon we observe to occur. We further confirm that all three ingredients: cooperative binding, cooperative unbinding at the tip, and a nucleation point are essential for our model to reproduce the experimental results. Fig. 6.8a shows a kymograph in the absence of cooperative binding, but where both a nucleation point and cooperative unbinding are present. Even at high \( N \) clusters do not form in the absence of cooperative binding. Cooperative unbinding is also critical. When we remove cooperative unbinding so that \( p_u^* = p_u \), though clustering does occur over regular time intervals, the population of motors at the tip is not enough to continuously hold the membrane tip in place even at \( N \) as high as 60, a value in the middle of the expected range on a membrane tube. The arrows in fig. 6.8b indicate points where there are no motors at the tip. If we lower the unbinding probability at the tip to simply account for crowding effects that reduce the unbinding rate, we find that the distinct timescale over which motor clusters form and arrive at the tip disappears as shown in the example in fig. 6.8c.

In the simulations in which we assume a nucleation point to be present at \( X \), we recover the experimentally observed linear relationship between arrival time and decay time for different values of \( N \). Fig. 6.9 shows the resulting average cluster arrival time vs. the average decay time at
Figure 6.8: **Verification of model components with simulations**

a) Simulation of a membrane tube with X and cooperative unbinding at the tip but without cooperative binding along the length of the tube. \( N = 100, \ L = 5 \mu m, \ X = 3 \mu m \) and \( p_u^* = 0 \). Clusters do not form. 

b) Simulation of a membrane tube with X and cooperative binding but without cooperative unbinding at the tip. \( N = 60, \ L = 5 \mu m, \ X = 2 \mu m \) and \( p_u^* = p_u = .01 \). Here, the population of motors at the tip is often not high enough to be able to hold the tip in place. An example of no motors at the tip is indicated by the arrow.

c) Simulation as in (b) without cooperative unbinding but with a lower unbinding probability at the tip: \( p_u^* = p_u = .0001 \). Here, the distinct timescale over which motors cluster and arrive at the tip disappears.
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the tip from simulations of different membrane tubes with varying $X$. The different symbols represent different $N$ and the arrows indicate an increase in $X$. The increases in $X$ are indicated in the table at the top of the fig. 6.9 where the numbers are distances in $\mu m$ behind the tip. We find that, at each $N$, an increase in $X$ results in an increase in both the average decay time at the tip and the average cluster arrival time.

![Graph](image)

Figure 6.9: **Average arrival time vs. decay time at the tip from simulations** Scatterplot of simulated data for different motor number ($N$), length ($L$) and $X$. The different symbols represent different $N$. For each $N$, moving $X$ to a position farther away from the tip (open symbols represent a larger $X$) results in a linear increase in timescales. The experimental data, indicated by the purple triangles, falls into the simulation regime.

The experimental data, indicated by purple triangles in fig. 6.9 fall into the same regime as the simulations for different $N$. Because the simulations that account for cooperative binding, cooperative unbinding at the tip and a nucleation point reproduce the experimental results, we suggest that motors in experiments are indeed recycled to make additional walking attempts to the membrane tube tip. The simulations provide an estimate for the cooperative binding probability of 0.24 and for the number of motors necessary to drive the system: $25 < N < 120$. 
6.4 Conclusion

We have shown that motors in stationary membrane tubes spontaneously create a recycling pattern of motor clusters that grow as they move towards the tip of the tube at typical timescales. Using Monte Carlo simulations, we show that cooperative binding can account for the formation of motor clusters. From the simulations we estimate a cooperative binding probability of 0.24 and a range for the concentration of motors necessary to drive the system to be between 25 and 120. We also find that, assuming a fixed point where the membrane tube meets the microtubule to be a nucleation point for motor clusters and cooperative unbinding at the tip of the membrane tube, a linear relationship between the average arrival time and tip decay time emerges.