Collective motor dynamics in membrane transport in vitro

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Chapter 1

Introduction

Key cellular processes such as cell division, internal cellular organization, membrane compartmentalization and intracellular transport rely on motor proteins. Motor proteins, ATP-based mechanoenzymes, actively transport cargo throughout the cell by walking on cytoskeletal filaments. Motors have been studied in detail on the single motor level such that information on their step size, ATP turnover rate, stall force, average run length and processivity are well known. However, in vivo, motors are often found working together, raising the question of how motors work together in transport. In vitro approaches to understand collective motor behavior that include gliding assays, bead transport, and DNA scaffolds have all provided much information about how motors coordinate stepping in order to transport cargo. However, in all of these experiments, motors are bound to a rigid surface. In their native environment, motors are bound to membrane material so that they can diffuse through a lipid bilayer, suggesting that their collective behavior may rely more on dynamic self-organization than experiments until now have allowed. In this thesis, an in vitro approach is presented to study collections of motors as they self-organize to actively transport membrane along microtubule tracks. [1]

[1] *Review in preparation*: Paige M. Shaklee, Thomas Schmidt and Marileen Dogterom. Collective motor dynamics in cargo transport.

1.1 Motor-driven transport

In cells, membrane-bounded vesicles and organelles are often transported over long distances (μm) along well-defined routes and delivered to particular locations. Diffusion alone cannot account for the rate, directionality, and acute destinations of these transport processes. The movement is driven by motor proteins: ATP-fueled mechanoenzymes that convert chemical energy into mechanical work.

Transport occurs over the cell's biopolymer tracks, namely microtubules (MTs) and actin filaments. There are two specific motor families responsible for long-range transport over MTs in cells: dyneins and kinesins. The MTs they traverse are constructed from tubulin heterodimers that associate head-to-tail giving rise to an intrinsic polarity in the MT.[1] Kinesin motors walk towards the dynamic "plus-end" of MTs (typically away from the cell's nucleus) while dynein motors walk in the opposite direction towards the "minus-end" of MTs. Studies inhibiting motor activity have shown that these two motors are essential for bidirectional transport inside of cells.[2] Both dynein and kinesin are processive motors: they take many steps before releasing from a MT. There are also nonprocessive motors that only take a single step before dissociating from a filament such as muscle myosin that interacts with actin filaments.[3] Both processive and nonprocessive motors are key players in intracellular transport and organization. The evidently critical role that motor transport proteins play *in vivo* led to questions about how these individual motor proteins are designed and how they function.

1.2 Single motor studies

Major advances in single molecule studies have provided a font of information about individual motor proteins. The structures of motor proteins are well known from biochemical isolation and DNA sequencing followed by techniques such as cryo-electron microscopy (cryo-EM), X-ray crystallography and nuclear magnetic resonance (NMR).[4,5] Fig. 1.1 shows a kinesin motor taking a step along a MT.[6] The motor binds to the MT via two globular head domains that are held together by a coiled coil

stalk. The the motor is bound to cargo via binding domains at the other end of the stalk (not shown in the image). Structural images of the precise conformation that motors maintain while bound to a MT in different nucleotide states have elucidated the way in which a motor's ATP cycle is coupled to their mechanical movement. These studies provide much information about the structure of motor proteins and the way in which they bind to a MT, but their dynamics have required different probes. A key step towards studying individual motor dynamics has been the isolation of motor proteins so that they can be examined in the absence of other proteins that might alter their behavior. Many microtubule motor proteins can be expressed in *E. coli*[7] and *S. cerevisiae*[8] and purified to use in *in vitro* experiments.

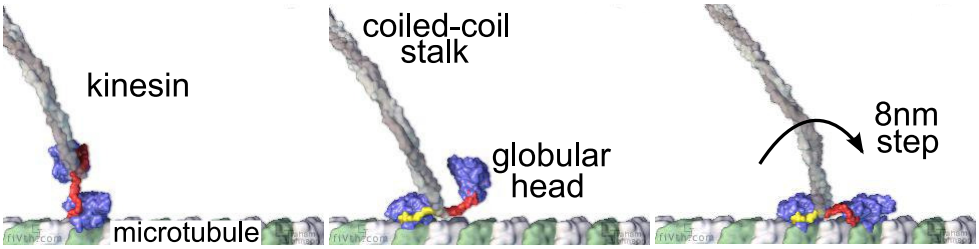

Figure 1.1: **Kinesin on a microtubule** a) Timeseries showing kinesin taking an $8nm$ step along a MT. The globular head domains bind to the MT and are held together by a coiled coil stalk. Cargo is bound at the other end of the stalk.[6]

Elegant experiments with optical traps have allowed enough spatial and temporal resolution to determine the stepsize of individual kinesin motors to be $8nm$.[9] Optical traps also provide force information in the pN range allowing many groups to determine that a kinesin's velocity decreases roughly linearly in response to load until stalling at a load of approximately 4 to $8pN$.[9–12] The distance a kinesin walks on a MT before dissociation, its runlength, has been shown to be $\approx 1\mu m$[13,14] and, at zero-load, the motors consume 1ATP/$step$ and walk at speeds up to $\approx 2\mu m/s$ *in vivo*[2] and $\approx 1\mu m/s$ *in vitro*.[15] Similar experiments have been performed for dynein.[16] Though processive like kinesin, dynein does not

always take uniform $8nm$ steps and often takes side or backsteps. Like dynein, the non-claret dysjunctional protein (ncd), responsible for MT bundling *in vivo*, moves to the minus end of MTs.[17,18] However, unlike dynein, ncd has been shown to be nonprocessive *in vitro*.[19,20] The motor has been used quite frequently as an attractive model to understand the ways in which nonprocessive motors function as compared to processive motors and we also review those findings here. Values for *in vitro* runlength, speed and ATP turnover rate of individual kinesins, dyneins and ncds under zero-load are shown in Table 1.1.

motor	runlength (nm)	speed (nm/s)	ATP^{-1}	stall force (pN)
kinesin	$800 - 1200$[13,14]	1000[15]	100	5[9–12]
dynein	$1000 - 1700$[16,21]	90[16]	–	7[22]
ncd	9[23]	≈ 12[23,24]	1.4[24]	–

Table 1.1: Table of *in vitro* runlength, speed and ATPase for individual microtubule motors: dynein, kinesin and ncd. It should be noted that the values for ncd are based on data for individual motors. Thus, the runlength represents the stepsize and the speed is the speed at which a motor takes a single step rather than the maximum speed that multiple motors can transport a cargo.

Though the wealth of single molecule information about individual motor proteins continues to grow, motors tend to work together.[25] Immunogold EM images of kinesins and dyneins on organelle fractions show motors that are grouped in clusters of two or more on membrane cargo and in many cases all the motors on the cargo are localized to a single cluster.[26–29] There is more and more evidence that cooperation between multiple motors in cargo movement is a key mechanism that cells use to regulate cargo transport.[30] Thus, recently, interest has increased in the area of collective motor dynamics.

1.3 From the individual to the collective

Many of the initial experiments to examine collective motor behavior have been performed *in vitro*. *In vitro* experiments are ideal experiments

to perform in order to ensure that no other external factors influence the dynamics observed under the microscope. Some of the very first *in vitro* experiments to examine collective motor behavior were gliding assays. In these assays, motors are bound by their tail to a glass surface, leaving their feet (or heads) free above (fig. 1.2b). When the motors encounter a

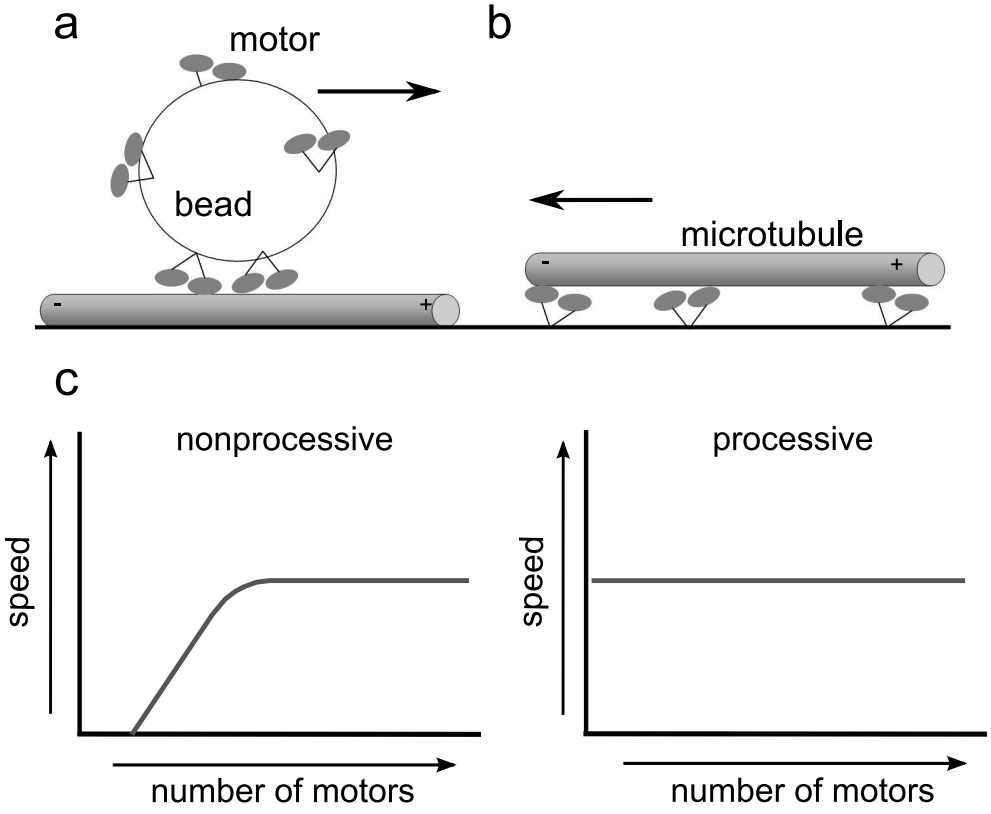

Figure 1.2: *In vitro* **motility assays** a) Bead assay: the bead moves in the same direction as the motors walk. b) Gliding assay: the microtubule glides along the surface in the direction opposite from the motor walking direction. c) The speed of MT gliding by nonprocessive motors is number dependent. However, for processive motors, the MTs are moved at the same speed regardless of the number of motors attached to the filament.

MT that is freely diffusing above in the bulk of a sample, they immediately bind to the MT. Because the motors are anchored in place, as they

walk, the MT glides across the surface. For processive motors, regardless of the number of motors and in the absence of load, the MTs glide at the same speed[31] suggesting that a certain degree of coordination between motors must exist.[3] In contrast, the MT gliding speed by nonprocessive motors *is* number dependent: for continuous gliding a threshold number of motors available to bind the filament is necessary. Thereafter gliding speed increases with motor density up to a maximum gliding speed (fig. 1.2c) that depends on the fraction of time a motor is bound to a filament during its ATP cycle: the duty ratio.[3] For example, ncd has a duty ratio of ≈ 0.08. The MT gliding speeds ncd motors can exhibit range from $12 - 160nm/s$[19,32] where $160nm/s$ is the saturation speed. In order for nonprocessive motors to transport a cargo, they must coordinate to form an effectively processive ensemble, such as the case of the Myo4p nonprocessive motor responsible for mRNA transport.[33,34]

The gliding assay has been used to determine the dynamics of various collective motor systems. Gliding assays with a mutant version of the minus-end directed ncd motor, NK11, have shown that motors can spontaneously change the direction in which they step resulting in bidirectional MT gliding.[35] Moreover, the assay has shown the force-mediated switching behavior of MTs that glide by competing plus-end directed kinesin and minus-end directed dynein[36] as well as by antagonistic kinesin-5 and ncd.[37] Coordination in stepping and binding/unbinding rates must all be uniquely coupled to regulate the motor ensembles. Major steps have been taken to advance this assay, so that it can be tailored using specialized surface chemistry to control the motor density on the surface and determine that a loose mechanical coupling between motors is necessary for efficient transport by motor ensembles.[38]

Because collections of motors are often used to transport micrometer-sized cargos *in vivo*, another typical assay to examine motor behavior is to attach motors to a bead *in vitro* and allow the motors to move the bead as they walk on underlying MTs (fig. 1.2a). Beads moved by kinesins move at constant speeds independent of motor number but run-length increases as more motors are available to interact with the MT.[30,39] In contrast, beads moved by dynein-dynactin complexes (dynactin serves

as a cargo binder) with a high number of motors tend to pause frequently and anchor the bead cargo at microtubule intersections.[40] Moreover, while individual dynein-dynactins can often display bidirectional movement, ensembles of dynein-dynactins move cargo unidirectionally (in both gliding and bead assays).[41]

In both the gliding assays and the bead assays, motors are randomly organized on a surface (flat glass surface or rounded bead surface) so that their position and orientation as well as relative distances from eachother are unknown. Recent experiments using DNA scaffolds to couple discrete numbers of motors at set distances have confirmed that multiple kinesins maintain longer runlengths than individuals while their speed does not vary.[42] When two motors are coupled (a distance of $50nm$ apart), though the transport speed does not vary, the unbinding rate of an individual motor is enhanced and cargo is no longer transported in discrete $8nm$ steps.[43]

1.4 Collective dynamics in membrane transport and tube pulling

In all of these experiments, motors are bound to a rigid surface. However, in their native environment, motors are bound to membrane material so that they can diffuse through a lipid bilayer, suggesting that motors' collective behavior may depend on the ability to assemble and freely rearrange configuration. This type of self-organization has not been allowed for in the experiments described so far. Preliminary experiments where collections of kinesins are attached to small oil droplets, a model system for small vesicles *in vivo*, exhibit the same transport characteristics as beads transported by multiple kinesins.[44] The physical properties of oil droplets are different from small vesicles made of membrane material, though, and the collective dynamics of MT motors in the absence of any other proteins on small vesicles has yet to be investigated.

An alternative model system to study collective membrane-bound motor dynamics is provided where functionalized kinesin motors are specifically attached to giant unilamellar vesicles (GUVs) and allowed to en-

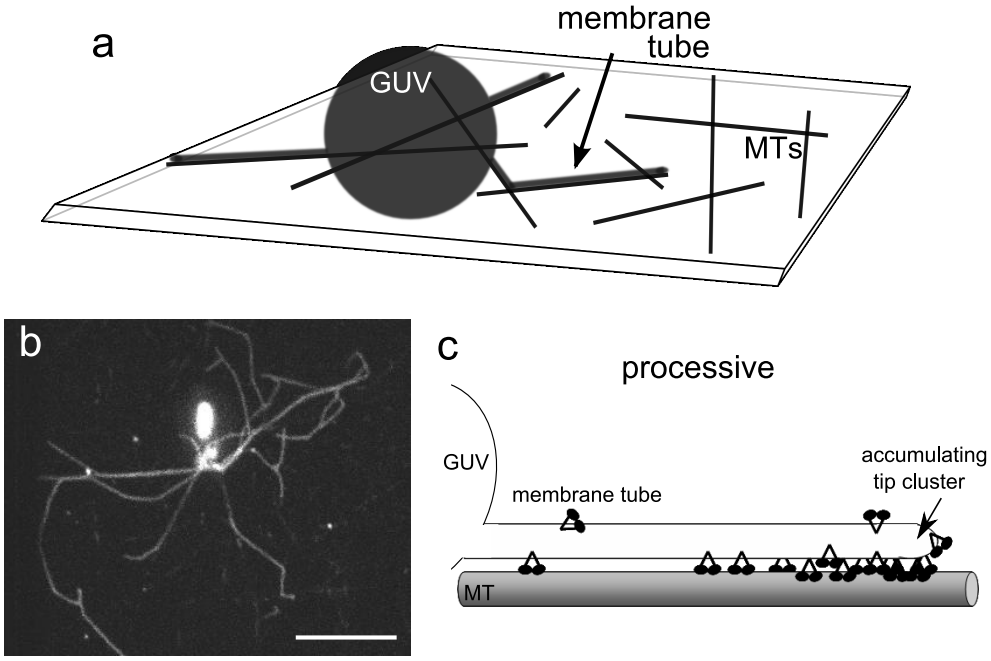

Figure 1.3: **Motors in membrane tubes** a) Schematic of experiments for tube pulling. A GUV coated with motor proteins sits on top of randomly arranged MTs on a glass surface. Motors walk along the MTs, pulling membrane material from the GUV with them to extract membrane tubes. b) Fluorescence image of an *in vitro* membrane tube network formed by kinesin motors on top of a mesh of unlabeled MTs. bar=$10\mu m$. c) Cartoon of processive motors in a membrane tube. The motors walk towards the tip at full speed, however, motors at the tip are slowed because of the tube pulling force so motors accumulate at the tip.

counter MTs on a surface have shown that with the simple addition of ATP, membrane tube networks are formed[45] (fig. 1.3a). An example of a network formed by kinesin motors is shown in figure 1.3b. The formation of these elaborate networks that mimic the dynamic membrane tube networks of the endoplasmic reticulum[46] relies on the cooperation of multiple motors. An individual motor cannot exert enough force to deform the GUV and extract a membrane tube, but collectively, clusters of motors can exert a force large enough to pull a tube. This force scales as $F_{\text{tube}} \sim \sqrt{\kappa\sigma}$, where κ is the membrane bending modulus and σ the surface tension.[47,48] The prediction that motors dynamically assemble and form a stable tip cluster to pull a tube[49] has been experimentally verified[50] and supported by a microscopic model.[51] Because the speed of motors at the tip of the tube is damped by the opposing tube-pulling force, motors that walk at full speed along the length of the membrane tube collect at the tip. Figure 1.3c shows a schematic cartoon of kinesin motors dynamically clustering at the tip of a membrane tube. Intriguingly, the unequal load felt by different motors in a membrane tube (where the highest load in the tube is at the very tip acting on the tip-most motor) may facilitate the clustering at the tip that is necessary to continuously move the tube.[52]

These experiments where motors can self-organize on a membrane cargo provide an experimental framework that allows us to explore many questions. We have seen that as more nonprocessive motors attach to a MT in gliding assays, the faster the MT glides. If nonprocessive motors are not rigidly coupled to a surface, and allowed to freely arrange on their cargo can they perform directed work for transport of e.g. membrane tubes? If so, how do the motors coordinate for transport? What are the dynamics of motors when they reach the end of a MT and can not pull a membrane tube any farther? How do motors of opposite directionality organize on vesicles to mediate bidirectional transport?

1.5 Contents of the thesis

In this thesis, I address these questions about motor collectivity in membrane transport. I use an *in vitro* approach where I attach motor proteins to membrane reservoirs, GUVs. When motors on the GUV encounter MTs on the surface, in the presence of ATP, motors self-organize to extract membrane tubes. I examine both the movement of the membrane tubes and the dynamics of the motors in the membrane tubes. Chapter 2 provides details on the materials and common methods used in these membrane tube experiments throughout this thesis.

I show, in chapter 4, the surprising result that membrane tubes can be formed by nonprocessive motors. Nonprocessive ncd motors not only extract membrane tubes from GUVs, but they also mediate bidirectional membrane tube dynamics. I present a model for this system and suggest that bidirectional tube movement is the eventuality of this system.[32]

Whereas in chapter 4, all the motor dynamics are inferred by examining membrane tube behavior, chapter 5 directly examines motor dynamics in membrane tubes. Motor dynamics at the MT-membrane tube interface are probed using the techniques of image correlation spectroscopy and fluorescence recovery after photobleaching. Nonprocessive motors bind to the MT over the entire length of the membrane tube, while as expected, processive motors accumulate at the tip of the tube.[50] I find a very small diffusion constant for motors at the MT-membrane tube interface suggesting that a constant high-density of motors is maintained to mediate the membrane tube dynamics seen in chapter 4.[53] The detailed derivations of the solutions for the autocorrelation function and fluorescence recovery profile in one dimension are written in chapter 3. The derivations are meant to describe membrane tubes which are approximated as one-dimensional lines for the cases of a) simple diffusion and b) where the particles in the system exhibit a directed motion.

Chapter 6 examines the recycling phenomenon that arises in nonmoving membrane tubes formed by processive motors. I present a model that proposes that cooperative binding[54] leads to the formation of clusters that walk towards the tip of a membrane tube. Cooperative binding combined with cooperative unbinding at the tip and a nucleation point

along the MT define a the recycling period. Based on comparison of the numerical results and experimental data I estimate a binding probability and concentration regime where the recycling phenomena occurs.[55]

Chapter 7 discusses future research directions that follow from the work in the rest of the thesis. In particular I present preliminary experiments and simulations examining the competition between dynein and kinesin motors in small vesicle transport *in vitro*.