

Dynamic Concentration of Motors in Microtubule Arrays

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We present experimental and theoretical studies of the dynamics of molecular motors in microtubule arrays and asters. By solving a convection-diffusion equation we find that the density profile of motors in a two-dimensional aster is characterized by continuously varying exponents. Simulations are used to verify the assumptions of the continuum model. We observe the concentration profiles of kinesin moving in quasi-two-dimensional artificial asters by fluorescent microscopy and compare with our theoretical results.

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The cytoskeleton is a network of polymers essential for the dynamic organization of many eukaryotic cells. Its function depends not only on protein fibers, but also on many accessory components [1]. Among these are motor proteins that reversibly bind to and walk along the surface of cytoskeletal polymers, consuming adenosine triphosphate as a source of energy. A natural consequence of this directed movement in organized fiber arrays is a nonuniform spatial distribution of the motors. *In vivo* microtubules are often observed in radial arrays, or asters, where all microtubule “minus” ends are at the center, and the “plus” ends are radiating outward. An aster of opposite polarity, in which kinesin moves inward, can also be formed *in vitro* [2].

In this Letter we present quantitative experiments on a quasi-two-dimensional aster, Fig. 1, formed from long microtubules polymerized between closely separated cover slips. We analyze the results of these experiments using a 2D transport-diffusion equation. The theory contains a number of approximations, due to angular averaging and projection from three to two dimensions. We verify that no major quantitative error is introduced by performing simulations in the full confined geometry. We also consider, theoretically, the case of one and three dimensions: The aster in one dimension corresponds to a tube in which microtubules are all oriented in the same direction. This is the case, for example, in the axons of nerve cells. As distinct motors move toward the plus or the minus end, we consider both cases of inward/outward directed motion.

Consider N immobile straight microtubules radially arranged in the available volume. Molecular motors are present which can exist in two different states, either attached to a filament or detached. Unattached motors diffuse freely, with a diffusion constant D . Attached motors move on their filament (radially in the aster geometry) at a velocity v . Positive values of v correspond to outward movement. Transitions between the two states are stochastic: The motor spontaneously detaches from the microtubule at an unbinding rate p^{off} (s^{-1}). Far from saturation, the number of binding events per second is proportional to the local concentration of free motors, and to the number of

available binding sites on the microtubules. If the concentration of free motors is expressed in molecules per cubic micrometers, and the available “quantity” of microtubules in micrometers, the constant of proportionality p^{on} has the dimension of a diffusion constant [3,4].

Let $b(r)$ and $f(r)$ be the concentrations of bound and free motors, respectively, at distance r from the center, averaged over all angles. Bound motors move radially at speed v , and create a convective flux $J_b = vb$. Unbound motors diffuse freely, creating a radial flux $J_f = -Ddf/dr$. If S is the surface area at distance r , there are $p^{\text{off}}Sbdr$ release events in the volume between the radii $[r, r + dr]$, and $p^{\text{on}}Nfdr$ attachments per second. We therefore obtain the coupled kinetic equations:

$$\begin{aligned} \frac{\partial b}{\partial t} &= -p^{\text{off}}b + \frac{p^{\text{on}}N}{S}f - \frac{1}{S} \frac{\partial}{\partial r} (J_b S), \\ \frac{\partial f}{\partial t} &= +p^{\text{off}}b - \frac{p^{\text{on}}N}{S}f - \frac{1}{S} \frac{\partial}{\partial r} (J_f S). \end{aligned} \quad (1)$$

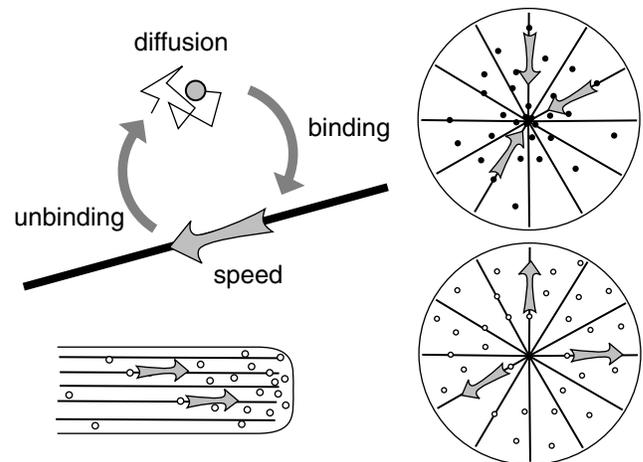


FIG. 1. In the presence of a microtubule array motors can move by free diffusion in a solution or by directed motion on microtubules. Movement of motors in an aster can lead to accumulation, if the motor moves inward (top right), or depletion, if the motor moves outward (bottom right). Accumulation also occurs in oriented parallel microtubule arrays (bottom left).

To find the steady state, we use the fact that the net flux $J_b + J_f$ is zero, implying $b = Dv^{-1}(df/dr)$. Substituting this result into Eq. (1), and denoting $f' = df/dr$ and $f'' = d^2f/dr^2$, we find in d dimensions

$$0 = f'' + \left(\frac{1}{\alpha} + \frac{d-1}{r}\right)f' - \frac{1}{r^{d-1}\beta_d}f, \quad (2)$$

$$b = \gamma f'.$$

The physical parameters in the problem have been reduced to

$$\alpha = v/p^{\text{off}},$$

$$\beta_1 = S_1 D/p^{\text{on}} N, \beta_2 = 2\pi h D/N p^{\text{on}}, \beta_3 = 4\pi D/p^{\text{on}} N,$$

$$\gamma = D/v, \quad (3)$$

where S_1 is the area of the tube in one dimension and h is the sample thickness in a quasi-two-dimensional geometry. α is the average distance that a motor moves on a microtubule before detaching. β_d characterizes the geometry of the aster, and γ determines the relative concentration of bound to free motors. Note that for inward movement, α and γ have negative values, while for outward movement all parameters are positive.

For the motor protein kinesin, experimental data provide values for the parameters in the model. The walking speed of kinesin without load is $v = 0.8 \mu\text{m s}^{-1}$ [5,6]. The unbinding rate p^{off} is obtained from the average distance that kinesin moves before detaching. Measured average run length, $\alpha = v/p^{\text{off}}$, is for kinesin in the range $0.4\text{--}1.5 \mu\text{m}$ [7–9]; we use $p^{\text{off}} = 1 \text{ s}^{-1}$. Direct chemical measurements of p^{off} [4] agree with this value. The binding rate p^{on} of the kinesin construct used in our experiment has not been directly measured. To estimate it, we assume that interaction between microtubules and motors is diffusion limited [10]: Measurements with kinesin's soluble dimeric motor domain [4] provide a value of $p_{\text{kin}}^{\text{on}} = 7.3 \mu\text{m}^2 \text{ s}^{-1}$, and its diffusion constant is $50 \mu\text{m}^2 \text{ s}^{-1}$ [11,12]. For single kinesin adsorbed on beads [9], the equilibrium constant for the binding convection (equal to $p^{\text{on}}/p^{\text{off}}$) provides $p_{\text{bead}}^{\text{on}} = 0.25 \mu\text{m}^2 \text{ s}^{-1}$, and a diffusion constant of $2 \mu\text{m}^2 \text{ s}^{-1}$ [13]. The ratios p^{on}/D are 0.14 and 0.12, respectively, and we use the averaged value $p^{\text{on}}/D = 0.13$. Finally, based on its molecular weight, we estimated a diffusion constant of $D = 20 \mu\text{m}^2 \text{ s}^{-1}$ [14], which yields $p^{\text{on}} = 2.6 \mu\text{m}^2 \text{ s}^{-1}$.

In a tubular, quasi-one-dimensional geometry, all microtubules are oriented in the same direction (Fig. 1), lower left. We find that the steady state profile for the motor concentration is exponential $f(r) \sim e^{r/a}$ where the distance a is a root of the equation $a^2 + a/\alpha - 1/\beta_1 = 0$. To relate this result to the situation of motors in a cell, we now consider a tube filled with oriented microtubules connected on one side to a large body. Motors have a concentration

which varies exponentially with the distance from the cell body. The concentration at the end of the tube is $e^{L/a}$ times smaller (or greater depending on the motor sense) than in the cell body, where L is the length of the tube. We estimated a for a cellular extension of a diameter of $2 \mu\text{m}$, and for parameters of the motor kinesin: If the extension contains 20 microtubules, then $a = 2 \mu\text{m}$; for the same tube containing a single microtubule, $a = 30 \mu\text{m}$. Therefore an unregulated kinesin (which can always bind and move) would be concentrated even in short extensions of a cell containing outward polarized microtubules. This is indeed observed *in vivo* for a kinesin heavy chain if it is overexpressed in the absence of the regulatory light chain [15].

Our experiments were performed in a quasi-2D geometry. We thus give particular weight to the theoretical analysis of this case which also presents some interesting theoretical features: Consider first a nonmotile binding protein ($v = 0$). At equilibrium, the unbound molecules are evenly distributed throughout the volume, $f(r) = \text{const}$, while the concentration of bound molecules is proportional to the local concentration of a microtubule so that $b(r) \sim 1/r$. Thus binding of motors induces their accumulation in the center, where microtubules are more concentrated.

For general speed v the solutions of the equations in 2D can be expressed in terms of Whitaker functions; however, simple analysis [performed by substituting $f(r) \sim r^{-x}$ in Eq. (2)] shows that the solution in the quasi-two-dimensional case is well approximated by power laws beyond the radius α^3/β_2^2 : $f(r) \sim r^{\alpha/\beta_2}$, and $b(r) \sim \gamma f(r)/r$. Thus the concentration profile of motors is characterized by an exponent which is a continuous function of the physical parameters. From the above expressions we find $\alpha = \pm 0.8 \mu\text{m}$, $1 < \beta_2 < 10 \mu\text{m}$, and $\gamma = \pm 60 \mu\text{m}$. The theory shows that in large asters (for kinesin, and a sample thickness of $9 \mu\text{m}$, asters with more than ~ 600 microtubules), most of the motors are trapped in the center, and very few motors are left elsewhere in the sample, effectively causing a dynamic ‘‘localization’’ [16]. The depletion from the aster center of a kinesin motor moving outwards is comparatively weaker. For small asters, motor concentration can be higher in the center, merely as a consequence of the binding of motors to microtubules. Total depletion is achieved only for large asters (of 1000 microtubules), for which outward transport overcomes the pure binding effect.

In three dimensions, the perturbation in the concentration due to the presence of the aster is significant only within a distance $\alpha/\sqrt{\beta_3}$ from the center. For large radii, $f(r) \sim (1 - r_0/r)$. We did not study this situation further due to the absence of experimental results.

To derive the convection-diffusion equations, we averaged over the directions transverse to the microtubules (both angularly, and over the thickness of the sample). This approximation breaks down experimentally in a thin

sample at the center of an aster where the geometry is three dimensional, or at large radial distances where the microtubules are too far apart for angular averaging. We thus performed simulations in a true confined geometry in three dimensions to check that no substantial errors are introduced in the two-dimensional theoretical description. In our simulations the aster is formed by microtubules of length $L = 50 \mu\text{m}$, with their plus end in the center of a cylindrical box of radius L , and of thickness $9 \mu\text{m}$. Each motor is characterized by its state (bound or free), and a vector (position). To compute the binding of motors, a rate p^+ and an interaction range ϵ were introduced, so that at each step, a free motor has a probability $p^+ dt$ to bind to any filament located at a distance (by projection) closer than ϵ ; this effectively corresponds to $p^{\text{on}} = p^+ \pi \epsilon^2$. At each time step $dt = 4.10^{-5}$ s, bound motors may detach with a probability $p^{\text{off}} dt$, and otherwise move radially by a distance $v dt$; free motors make random steps with $\langle dx^2 \rangle = 3Ddt$. The motor parameters were taken to mimic kinesin (see above), with the additional value $\epsilon = 50$ nm [9], and $p^+ = 312 \text{ s}^{-1}$ (which yield the correct value for p^{on}). Other choices of ϵ and p^+ conserving p^{on} gave similar curves. The agreement between simulation and theory, Fig. 2 confirms that our 2D analytical approximations are faithful to the thin 3D situation.

We now turn to our experimental observations on confined quasi-two-dimensional samples: Using fluorescent microscopy we measured the kinesin distribution in asters with the two possible polarities. These asters have either the plus or the minus ends of the microtubules in the center [2,14], and take about 30 min to form. All data presented here are extracted from two identically prepared samples,

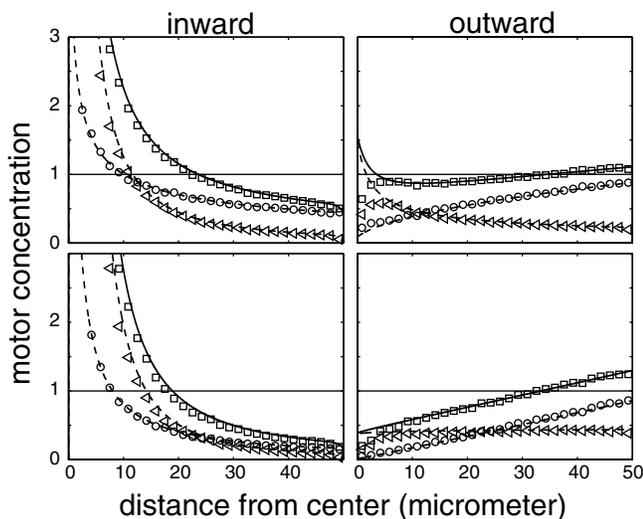


FIG. 2. Simulated bound (triangles), free (circles), and total = bound + free (squares) motor concentrations and 2D theory (lines), for kinesin moving inward (left) or outward (right) in asters having either 300 (top) or 600 (bottom) microtubules. As expected some deviations are seen near the center of the aster due to finite sample thickness implemented in the simulation.

in which many asters of various sizes formed. We measured 115 regular asters with an automatic epifluorescence microscopic setup (Zeiss axioplan 2 with Olympus $100\times$ oil-immersion objective). We detected the motors (labeled with the fluorophore fluorescein) and the microtubules (labeled with rhodamine) independently. Digital pictures were taken with a 12-bit charge-coupled device camera (Hamamatsu C4742-95, 1280×1024 pixels). The camera is linear, and unsaturated pixel values reflect the relative quantity of protein in the imaged region. The sample thickness was $9 \mu\text{m}$.

The center of the aster and the profiles of fluorescence intensity are calculated from the image. A common background pixel value was subtracted from the motor profiles, which are then normalized. An exponent is obtained by fitting the profile in the range $1.5\text{--}20 \mu\text{m}$ (the data below $1.5 \mu\text{m}$ are noisy). To measure the number of microtubules in the aster, we fit the profile of microtubule fluorescence to the function $(M/r + B)$, where r is the distance from the center. B is a background, and M is proportional to the number of microtubules. Calibration was done by manually counting the microtubules in five asters. The $1/r$ profile corresponds to an homogeneous aster of long microtubules. Experimentally the asters are not perfect (some, as in Fig. 3 left, are not well focused). When the fit of the microtubule profile to $1/r$ is poor, we have no reason to expect the theory to apply. These asters are plotted with a different symbol in Fig. 4.

We measured the distribution of kinesin in asters containing different numbers of microtubules. Three typical examples are given in Fig. 3. Motor profiles of individual asters are rather well fitted by a power law. They are almost linear on a log-log plot, and steeper for bigger asters, Fig. 4, inset. Plotting the exponent of the motor profiles as a function of the number of microtubules extracted from the microtubule profiles allows us to compare directly experiments and theory (see Fig. 4). The data points, each representing one aster, are scattered around the theoretical

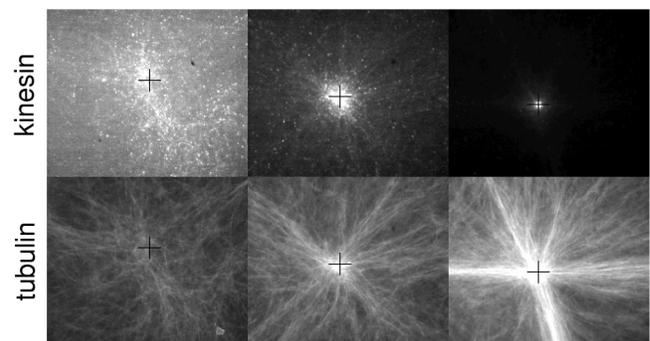


FIG. 3. Motor and microtubule distribution in experimental asters with a different number of microtubules: Fluorescence images ($90 \times 70 \mu\text{m}$) obtained for the motors (top), and for the microtubules (bottom). The crosses mark the computed measured centers. The intensities of the images are here scaled by different factors.

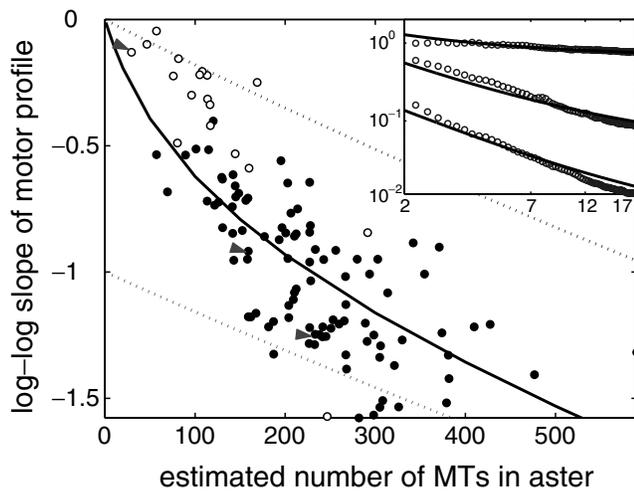


FIG. 4. The effective exponent of the kinesin concentration profile becomes more negative with the increasing numbers of microtubules in the aster (continuous line: 2D theory). Filled symbols correspond to regular asters for which the radial microtubule density falls as of $1/r$. Open symbols correspond to irregular asters where the density is inconsistent with a law in $1/r$. Arrow heads point to asters shown in previous figures. Inset (log-log): Experimental (circles) motor concentration profiles, as extracted from the pictures shown in Fig. 3, and 2D-theoretical curves computed for the measured number of microtubules (27, 148, and 231) in the aster.

curve, reflecting the heterogeneity of the asters. However, the major trend in the exponent is correctly predicted by the theory, so that denser asters are characterized by a larger localization exponent for the motors. We also imaged kinesin moving outwards in asters of normal polarity [2], but the signal was too dim to extract a reliable profile (the predicted kinesin profile in this situation is rather flat).

Accumulation or expulsion of molecular motors in asters can have important functional implications in biology. For instance within a spindle made of two interacting asters of microtubules, minus-ended motors could concentrate at the poles while plus-ended motors would be excluded from the same regions. This could contribute to the mechanism of spindle assembly, and/or to its mechanical stability. Interestingly we find that kinesin accumulates in asters of 300–1000 microtubules, which is comparable to the number of microtubules present in spindle asters of most animal cells. However, the geometry and motors of the spindle are not the ones studied here.

We did not consider the regulation of motor activity: In our study motors can always bind and move on filaments. Cells use a variety of processes to counterbalance the impact of motor transport on their localization. For example, the folding of kinesin into a nonmotile conformation, in the absence of a cargo [11,17,18], dampens the transport-induced localization. The recombinant kinesin fraction used in our experiment lacks this capacity. Even with this

partial inhibition, the movement of loaded kinesin brings them to places from which they have to be recycled. Additional regulation mechanisms include local synthesis and degradation of the motors, involvement of motors of different directionality transporting each other, etc. On the other hand, we can also imagine situations in which the unregulated localization of motors resulting from their movement can have interesting consequences.

In summary, motor movements on microtubules can effectively cause their “compartmentalization.” The theory provides an understanding of the influence of all motor kinetic parameters, and of the geometric properties of the microtubule array.

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