Biophysical Letter

Quantifying Protein Diffusion and Capture on Filaments

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ABSTRACT The functional relevance of regulating proteins is often limited to specific binding sites such as the ends of microtubules or actin-filaments. A localization of proteins on these functional sites is of great importance. We present a quantitative theory for a diffusion and capture process, where proteins diffuse on a filament and stop diffusing when reaching the filament's end. It is found that end-association after one-dimensional diffusion is the main source for tip-localization of such proteins. As a consequence, diffusion and capture is highly efficient in enhancing the reaction velocity of enzymatic reactions, where proteins and filament ends are to each other as enzyme and substrate. We show that the reaction velocity can effectively be described within a Michaelis-Menten framework. Together, one-dimensional diffusion and capture beats the (three-dimensional) Smoluchowski diffusion limit for the rate of protein association to filament ends.

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The catalytic activity of enzymes is often restricted to specific binding sites. The ends of microtubules (MTs) for example are binding sites for a plethora of MT-associated proteins (MAPs) (1). At MT ends, MAPs can catalyze biochemical processes (2), or serve as substrates for other enzymes. This makes an efficient association of MAPs to MT tips important. Experiments suggest that one-dimensional diffusion of MAPs on MTs facilitates tip-targeting (3,4). This idea goes back to the concept of "reduction in dimensionality" suggested by Adam and Delbrück (5) and has been largely applied (6,7). However, a quantitative understanding of tip-binding mediated by diffusion on the filament and subsequent capture at the tip has remained elusive (3,8–16).

Here we show that capturing at the tip is crucial for tip-localization of proteins. We present a theory where diffusion and capture is accurately quantified with an effective association rate constant and provide a result that depends only on experimentally accessible parameters. For proteins that are enzymatically active at filament ends, our theory predicts that diffusion and capture leads to an enhancement of the enzymatic reaction velocity due to stronger tip-localization. We observe that the reaction velocity in dependence of the enzyme concentration closely follows a Michaelis-Menten curve and quantify the contribution of one-dimensional diffusion to tip-localization and enzymatic processes downstream thereof.

To model the diffusive motion of proteins on a filament, we consider a one-dimensional lattice of length l with lattice spacing $a = 8.4$ nm (Fig. 1 *A*). The lattice corresponds to a single protofilament of a stabilized MT in the absence of dynamic instability. Proteins perform a random walk on the lattice with a hopping rate ϵ, the diffusion constant is $D = \epsilon a^2$. Each site can be occupied by only one protein, as the system is an exclusion process (17). Proteins attach to and detach from the lattice at rates $\omega_{on}c$ and ω_{off}, respectively, where c is the concentration of proteins in solution. The tip of the MT is represented by the first lattice site in our model. To account for its particular structure, different on- and off-rates are assumed there, expressed as $k_{on}c$ and k_{off}. Proteins that bind to the tip are captured, i.e., not allowed to hop on the lattice, but still may detach into solution. This important condition is a critical difference between our model and previous approaches ((3,16); and see also the Supporting Material).

The central goal of this letter is to quantify the relative contributions of diffusion and capture (tip-attachment after diffusion on the lattice) and end-targeting (attachment after diffusion in solution) (Fig. 1 *B*) to tip-localization. To this end we calculated the probability to find a protein at the end of a protofilament (the tip density ρ_+). In the absence of diffusion and capture, the Langmuir isotherm is obtained,

$$\rho_+(c) = \frac{c}{K + c}, \tag{1}$$

where $K = k_{off}/k_{on}$ is the dissociation constant of the protein at the tip. However, as noted previously (3,4), such a model is incomplete as it does not account for the additional

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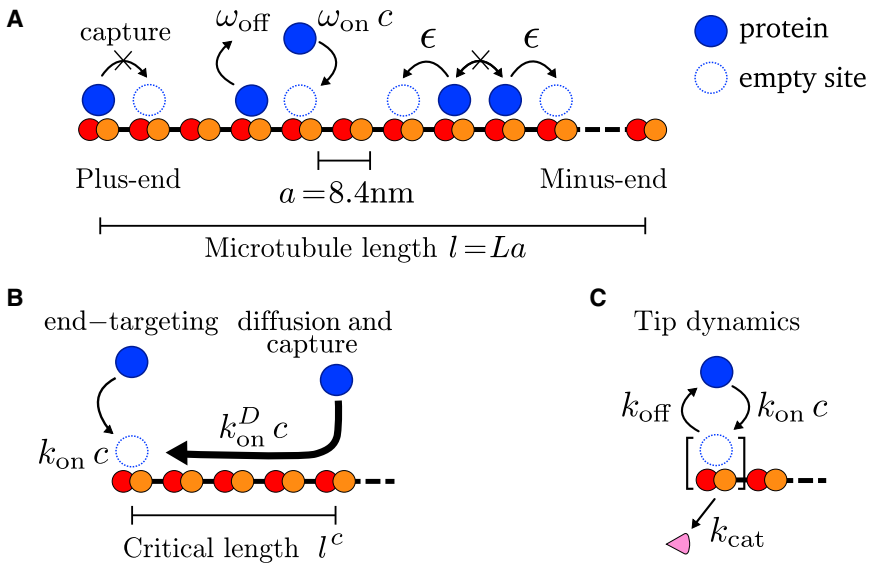

FIGURE 1 **(*A*) Schematic of a microtubule (MT) with diffusive tip-binding proteins. In the bulk of the lattice, proteins attach to empty sites and detach. Proteins hop to neighboring sites but obey exclusion. At the plus-end, particles are captured. (*B*) Illustration of direct tip-attachment from solution and via diffusion and capture. (*C*) Proteins bind reversibly at the plus-end. While a protein is attached there, a reaction is catalyzed at rate k_{cat}. To see this figure in color, go online.**

protein flux along protofilaments mediated by diffusion and capture. We have analyzed this flux by stochastic simulations of the model (Fig. 1). Surprisingly, we find that over a broad range of concentrations c, the additional protein current to an unoccupied reaction site J^D effectively obeys first-order kinetics, i.e., $J^D = k^D{}_{on}c$ (Fig. S2 in the Supporting Material). This observation implies that despite the complexity of the diffusion-reaction process one approximately retains the functional form of the Langmuir isotherm. Accounting for the diffusion-capture contribution to the rate of protein attachment leads to an effective dissociation constant,

$$K^{\text{eff}} = k_{\text{off}}/\left(k_{\text{on}} + k_{\text{on}}^{D}\right). \tag{2}$$

We have calculated the diffusion-capture rate k_{on}^D analytically, by exploiting the observed approximate linear reaction kinetics. We find

$$k_{\text{on}}^{D} = \frac{\omega_{\text{on}} D/a^2}{\omega_{\text{off}} + \sqrt{\omega_{\text{off}} D/a^2}}. \tag{3}$$

Refer to the Supporting Material for a detailed derivation of Eqs. 1–3. Together, Eqs. 1–3 comprise an effective theory for the association of proteins to the tip, which accounts for direct end-targeting as well as the diffusion-capture process. With Eq. 3, we are able to quantitatively predict the relative contribution of diffusion and capture to tip-binding for different proteins that diffuse on filaments. The results are shown in Fig. 2: 90–99% of molecules bind to the tip through one-dimensional diffusion, given they follow diffusion and capture.

Tip-localization due to diffusion and capture as predicted by our theory has important implications for enzymatically active proteins. We extended the model to investigate enzymatic reactions at the MT tip, where the protein-tip complex catalyzes a product at rate k_{cat} (Fig. 1 *C*). In detail, we assume that the protein does not leave the tip after catalyzing a reaction, but only through detachment into solution. These model assumptions are consistent with filament polymerizing enzymes that act processively, such as XMAP215 for MTs (9,10), and VASP (15) and formins (18) for actin filaments. The assumption of a constant length l in our model is excellent if the rate of diffusion is fast compared to the polymerization rate.

With the above model assumptions, the reaction velocity v is determined by the tip density, $v = \rho_+ k_{\text{cat}}$. We can apply our previous results, Eqs. 1–3, to obtain

$$v(c) = k_{\text{cat}}\,\rho_+(c) = \frac{k_{\text{cat}} c}{K^{\text{eff}} + c}. \tag{4}$$

The above equation is reminiscent of a single-molecule Michaelis-Menten equation (19,20) when K^{eff} is reinterpreted as the Michaelis constant and substrate and enzyme concentrations are interchanged. In this way, our theory constitutes an effective Michaelis-Menten theory, accounting for end-targeting and diffusion and capture. Instead of solving a complex many-body problem, it suffices to apply a mathematical framework that is analogous to (single-molecule) Michaelis-Menten kinetics. The details of diffusion and capture are accurately included in the effective on-rate

$$k_{\text{on}}^{\text{eff}} = k_{\text{on}} + k_{\text{on}}^{\text{D}}.$$

This result is in accordance with experimental results for several enzymatically active proteins where Michaelis-Menten curves were observed for the reaction speed depending on the enzyme concentration (8,9). Inspired by the processive (de)polymerase activity of (MCAK) XMAP215, we assume that enzyme and substrate are not

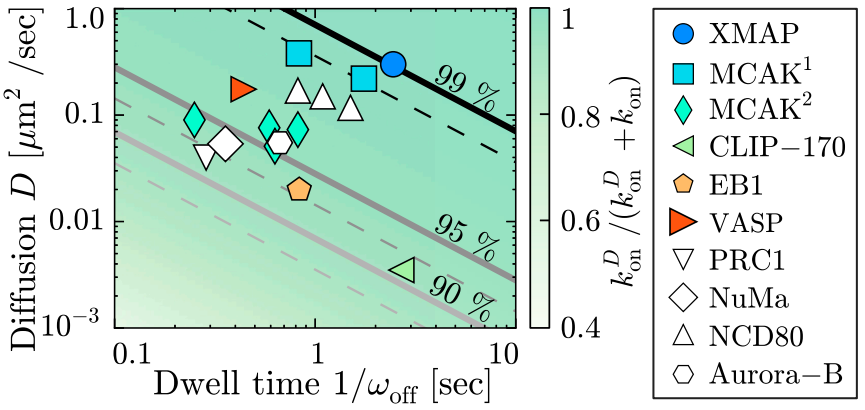

FIGURE 2 The model predicts the relative contribution to tip localization of proteins due to diffusion and diffusion and capture (*color code* and *solid lines*), $k_{\rm on}^D/(k_{\rm on}^D + k_{\rm on})$ with $k_{\rm on} = \omega_{\rm on}$ (*dashed line* for actin: $a = 6$ nm). Proteins that are captured at the filament end (*solid symbols*) and proteins where evidence for capturing is lacking (*open symbols*) are shown. Proteins that in addition have a direct enzymatic activity at the filament end are XMAP215 (9,10), MCAK[1] (3), and MCAK[2] (8) on MTs, and VASP on actin filaments (15; S.D. Hansen and R.D. Mullins, University of California San Francisco School of Medicine, personal communication, 2014). There exist also proteins that diffuse on MTs without enzymatic activity at MT ends, but with roles downstream of tip-localization, e.g., in the protein network of MT tips (1): Ndc80 (11), CLIP-170 (12), NuMA, PRC1, EB1 (13), and Aurora-B (14). To see this figure in color, go online.

decomposed in the reaction step. However, it is straightforward to include a decomposition in the theory: the corresponding effective dissociation constant would read

$$K^{\rm eff} = \left(k_{\rm off} + k_{\rm cat}\right)/k_{\rm on}^{\rm eff}.$$

Our analytical results, Eqs. 2–4, agree well with simulation results of the stochastic model, as shown in Fig. 3, *A* and *B*. We find that the diffusion and capture mechanism dramatically increases $k_{\rm on}^{\rm eff}$ and thereby reduces the effective dissociation constant typically by more than one order of magnitude, e.g., for XMAP215 we find $K^{\rm eff} \approx 10^{-2}\ K$ (see Table S1 in the Supporting Material for parameter values). In the case of long dwell-times $\omega_{\rm off}^{-1}$ and fast diffusion ϵ, $K^{\rm eff}$ reduces to a particularly simple form

$$K^{\rm eff} = \left(k_{\rm off}/\omega_{\rm on}\right)\Big/\sqrt{\epsilon/\omega_{\rm off}}, \qquad (5)$$

where the denominator is the square-root of the average number of diffusive steps a protein performs on the filament.

Note that one-dimensional diffusion without capturing (16) does not lead to a particle flux on the filament (Fig. S4), and hence the reaction velocity is not increased (Fig. 3 *A*). Further, the particle flux might be limited by the length of the filament: below a threshold length l^c (which is smaller than typical in vivo lengths of MTs), we observe a length-dependent behavior of the reaction velocity (Fig. S3), where our theory is not valid.

Our analysis reveals diffusion and capture as an efficient mechanism to circumvent the diffusion limit for the rate of end-targeting: Smoluchowski's theory of three-dimensional diffusion physically limits the rate of direct tip-attachment from solution (21). As shown here, one-dimensional diffusion along a filament and subsequent capture at the filament end overcomes this limitation. This has been shown experimentally for MCAK (3). Our work provides an applicable theory for reaction kinetics facilitated by diffusion and capture: specific parameter values for diffusion, tip-association, and dwell times can be accounted for (see Eqs. 3 and 4). Employing a broader perspective, our results may also be

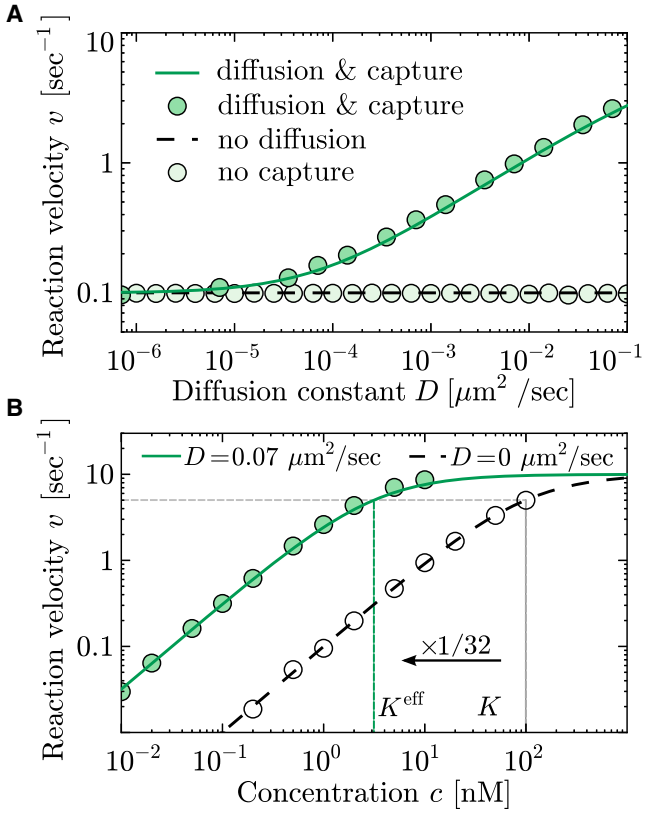

FIGURE 3 (*A*) Comparison of the reaction velocity with (*solid*) and without (*dashed*) lattice diffusion and with and without capturing at the tip (*circles*, simulation data; *lines*, analytic results). (*B*) Reaction velocity v in dependence on the protein concentration c. Analytic results (*lines*) are confirmed by simulation data (*circles*). Parameters are $L = 1000$, $\omega_{\rm off} = k_{\rm off} = 1\ {\rm s}^{-1}$, $k_{\rm cat} = 10\ {\rm s}^{-1}$ $\omega_{\rm on} = k_{\rm on} = 0.01\ {\rm s}^{-1}\ {\rm nm}^{-1}$, and $c = 1$ nM. To see this figure in color, go online.

applicable to other systems where one-dimensional diffusion is important (6), including transcription factor binding on DNA (22).

SUPPORTING MATERIAL

Supporting Materials and Methods, four figures, and one table are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(15)00063-6.

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