Microtubules (MTs) are essential components of the eukaryotic cytoskeleton that serve as “highways” for intracellular trafficking. In addition to the well-known active transport of cargo by motor proteins, many MT-binding proteins seem to adopt diffusional motility as a transportation mechanism. However, because of the limited spatial resolution of current experimental techniques, the detailed mechanism of protein diffusion has not been elucidated. In particular, the precise role of tubulin tails and tail modifications in the diffusion process is unclear. Here, using coarse-grained molecular dynamics simulations validated against atomistic simulations, we explore the molecular mechanism of protein diffusion along MTs. We found that electrostatic interactions play a central role in protein diffusion; the disordered tubulin tails enhance affinity but slow down diffusion, and diffusion occurs in discrete steps. While diffusion along wild-type MT is performed in steps of dimeric tubulin, the removal of the tails results in a step of monomeric tubulin. We found that the energy barrier for diffusion is larger when diffusion on MTs is mediated primarily by the MT tails rather than the MT body. In addition, globular proteins (EB1 and PRC1) diffuse more slowly than an intrinsically disordered protein (Tau) on MTs. Finally, we found that polyglutamylation and polyglycylation of tubulin tails lead to slower protein diffusion along MTs, although polyglycylation leads to faster diffusion across MT protofilaments. Taken together, our results explain experimentally observed data and shed light on the roles played by disordered tubulin tails and tail modifications in the molecular mechanism of protein diffusion along MTs.

Significance

Diffusion is a common cellular transport mechanism, which often takes place in lower dimensionality such as of proteins along DNA or on microtubules that are crucial for various biological processes. Although traffic on microtubules is performed by motor proteins, these proteins and others also diffuse on microtubules spontaneously without adenosine triphosphate consumption while engaged in essential cellular transport processes, such as cell division and neuronal development. Here, we explored how the periodicity of the α and β tubulins and their electrostatic properties govern the speed and mechanism of protein diffusion along microtubules. Particularly, using various computational models, we quantified the effect of the negatively charged disordered tails and their diverse posttranslational modifications on protein–microtubule interactions and dynamics.

Author contributions: L.S.B. and Y.L. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no competing interest.

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Utilize energy from adenosine triphosphate hydrolysis to move along MTs. In addition, it was shown that polyE acts as a rheostat to control the activity of spastin, an MT-severing enzyme important in neurogenesis and axon regeneration (20). An excess of polyE was also shown to perturb neuronal transport (21), and several links between various tubulin PTMs and human disease have been reported (17).

The existing evidence for the regulatory role that tubulin tails and their modifications play with respect to proteins that interact with MT gives rise to the possibility that the characteristics of tails may regulate the interactions of a broader class of proteins with MTs. One intriguing example is of proteins that use lattice diffusion (namely, a random walk driven by thermal energy only) to translocate along MTs and reach various target sites. The diffusing proteins exhibit a broad range of molecular properties, including with respect to dimension and degree of flexibility. For example, EB1 (22), the Dam1 (23) complex, XMAP215 (24), and kinesin 13 (25) (MCAK) use lattice diffusion to reach the plus end of MTs, where they are involved in tight regulation of MT length, which is crucial for cell division and neuronal development. By contrast, PRC1 (22), which cross-links two antiparallel MTs at way of forming spindle midzones at anaphase (26), and the intrinsically disordered protein Tau (27), which increases the stability of neuronal MTs, diffuse along the MT lattice, although they do not need to reach a specific end. Interestingly, some motor proteins combine diffusional and directed motility to diffuse in a biased manner along the MT lattice (28-31) or to side step across MT protofilaments (32, 33), a mechanism that may enable overcoming roadblocks on crowded MT lattice.

The diverse structures, sizes, and biological functions of the diffusing proteins suggest that lattice diffusion is a broadly applicable transportation mode for proteins that translocate along MTs. In order for proteins to diffuse along MTs, their affinity must be precisely tuned in that high affinity detracts from mobility on the lattice, whereas weak affinity detracts from binding the lattice. There is evidence that electrostatic interactions play a central role in this delicate balance (34). An increase in salt concentration leads to an increase in the diffusion coefficient of some proteins (22, 27) as does the enzymatic removal of the negatively charged tails (25, 27). These observations are somewhat expected since the surface of the MT lattice is negatively charged, and the surface of many MT-binding proteins includes both negatively and positively charged residues (SI Appendix, Fig. S1) (35). In this respect, diffusion of proteins along MTs is reminiscent of the one-dimensional diffusion of proteins along the negatively charged double-stranded DNA (36). However, in some cases, removal of tubulin tails has been found not to affect diffusion (37, 38), which gives rise to the possibility that electrostatic interactions alone cannot explain the diffusional motility of proteins along MTs (35).

Research thus far about protein diffusion along MTs, mostly using single-particle tracking by total internal reflection fluorescence (TIRF) spectroscopy, has shown that lattice diffusion is a transportation mode that proteins use broadly to translocate along MTs. However, because of the limited spatial resolution of TIRF spectroscopy (typically limited to the size of a fluorescently labeled protein, ~1 to 2 nm) and challenges in purification of tubulin with well-determined isoforms and PTMs (39, 40), the molecular details of the mechanism of protein diffusion along MTs are still not fully understood. Most importantly, it is not known how the structural characteristics of the MT, including tubulin tails and their modifications, affect protein diffusion.

In this study, we use coarse-grained (CG) molecular dynamics (MD) simulations to study the molecular mechanism of the diffusion of three different proteins (EB1, PRC1, and Tau) along MTs. In particular, we study how the disordered tails and their modifications (i.e., polyE and polyG) affect the mechanism and the speed of diffusion. This is achieved by studying a variety of MT variants in which the electrostatic properties of the tubulin bodies and tails were manipulated. Our study not only reproduces the experimentally determined diffusion coefficients but also, provides a detailed biophysical understanding of the molecular mechanism of protein diffusion along MTs and predictions as to the effect that PTMs may have on protein diffusion on MTs.

**Results**

**Electrostatic Interactions Control Protein Diffusion along MTs.** To study the dynamics of protein diffusion along MTs, we constructed a CG C_α-based model of a 4 x 6 MT lattice that includes the MT body with tubulin tails (Fig. 1 A and B; Methods has details). While the MT body remained rigid throughout the simulations,
the tails were flexible (Fig. 1C). The degree of tail flexibility was validated against their flexibility in atomistic MD (Methods and SI Appendix, Fig. S2). The diffusing proteins (EB1, PRC1, and Tau) were modeled using a native topology-based potential, and the time evolution of the interaction between the proteins and MT was studied using the Langevin equation (Methods has details).

We studied the diffusion of each of the three different MT-binding proteins on three types of MT lattice: 1) MT with the wild-type (WT) tails of both α and β tubulin (Methods has the exact definition), 2) MT without α/β tails, and 3) MT tails modified by the addition of polyglutamate (polyE) chains that were 10 amino acids long (Fig. 2A and SI Appendix, Figs. S7 and S8 and Movies S1–S3). Each system was simulated at salt concentrations in the range from 0.01 to 0.07 M. For EB1, we found that increasing the salt concentration leads to a gradual increase in the diffusion coefficient along protofilaments ($D_y$, longitudinal diffusion) in systems with no tails (Fig. 2B, blue circles) or with WT tails (Fig. 2B, red circles). When the tails are polyglutamylated (Fig. 2B, orange circles), the increase in salt concentration does not lead to a significant increase in the $D_y$ values. Similar results were observed for the lateral diffusion of EB1 on MTs (SI Appendix, Fig. S3). Increasing the salt concentration also leads to increased dissociation events for EB1 from the MT, and therefore, the fraction of EB1 bound to MT decreases. The effect of salt concentration on the nonspecific affinity of EB1 to MT is pronounced for MT with and without tails but is small for polyglutamylated MT (Fig. 2C).

When comparing the dynamics of the three MT-binding proteins (EB1, PRC1, and Tau) with MTs, it seems that EB1 diffuses with a $D_y$ of \(\sim 0.03 \text{ } \mu\text{m}^2/\text{s}\), which is similar to the experimental value. PRC1 diffuses with a $D_y$ of \(\sim 0.014 \text{ } \mu\text{m}^2/\text{s}\), which is within a factor of three of the experimental result (22). The $D_y$ value of the disordered protein Tau is \(\sim 0.44 \text{ } \mu\text{m}^2/\text{s}\), an order of magnitude larger than for the structured proteins (EB1 and PRC1), in accordance with the experimental results (SI Appendix, Figs. S3 and S4) (27). We note that similar $D$ values are obtained when estimated by mean square displacement or by kinetic analysis (SI Appendix, Fig. S4). For the three proteins, the presence of tails slows down longitudinal and lateral diffusion ($D_x$, diffusion across protofilaments) and increases the nonspecific affinity (SI Appendix, Fig. S3). PolyE of the tails enhances these effects. Our results are also consistent with experimental studies that examined the effect of increasing salt concentration and the removal of tails on diffusion (25, 27). A plausible explanation of our results (35) is that increasing salt concentration leads to screening of electrostatic interactions and hence, to weaker interactions between the diffusing protein and MT and therefore, faster diffusion. Hence, we conclude that electrostatic interactions are a key molecular force that drives protein diffusion along MTs. Moreover, the observation that diffusion is slower in the presence of tails supports the possibility that interactions between tails and the diffusing protein are crucial.

**Tubulin Tails Create Molecular “Lanes” for Protein Diffusion.** To better understand the effect of MT tails on protein diffusion, we focused on the diffusion of EB1 on MT lattices with tail components that have been manipulated. Four types of tails are considered: 1) WT tails, 2) electrostatically neutralized β tails and charged α tails, 3) electrostatically neutralized α tails and charged β tails, and 4) electrostatically neutralized α and β tails. In Fig. 3, we show two-dimensional heat maps of the probability of finding the center of mass of EB1 on MT lattices of the different systems. The heat maps are oriented such that the protofilaments are aligned along the y axis, and each row in the grid corresponds to either an α or β tubulin monomer.

For the system with WT tails (Fig. 3A), we found that EB1 diffuses in discrete steps of size \(\sim 8 \text{ } \text{nm}\) (vertical grid square is
4 nm), which corresponds to the size of a tubulin dimer. In this system, EB1 diffuses both along protofilaments and across them (side stepping). In the system with neutral α + β tails (Fig. 3D), EB1 diffuses in discrete steps of size ~4 nm, which corresponds to the size of a tubulin monomer. Diffusion occurs mostly along protofilaments, and side stepping is less frequent in this system. In the systems with neutral α tails (Fig. 3C) or neutral β tails (Fig. 3B), side stepping is the more populated diffusion mode. One interesting difference between the systems in which only one type of tail (the α or β tail) bears charges is the lane the protein uses for side stepping. In the neutral β tails system, protein diffusion takes place between two rows of α tubulin; however, in the system with neutral α tails, there is a clear 4-nm downshift, and protein diffusion takes place between two rows of β tubulin.

To obtain a deeper understanding of the importance of the precise composition of the tails, we constructed several intermediate systems. Fig. 3 A and D–F (visually linked by dotted arrows) shows the results of gradually shifting the MT system from one bearing WT-like tails (Fig. 3A) through intermediate systems in which two (Fig. 3E) or six (Fig. 3F) charged residues were neutralized from both the α and β tubulin tails of the WT MT lattice to a lattice bearing only neutralized tails (Fig. 3D).

Fig. 3 A, D, G, and H (visually linked by dashed arrows) represents contrasting systems comprising WT tails (Fig. 3A) compared with solely α (Fig. 3G) or solely β (Fig. 3H) tubulin tails compared with entirely neutralized tails (Fig. 3D). The heat maps for solely α or β tails (Fig. 3 G and H) are more uniform than those produced when only one type of tail is neutralized (Fig. 3 B and C) or when only some residues on both tails are neutralized (Fig. 3 E and F), and the discretization that is clear in Fig. 3 A–D seems to diminish. Hence, it is suggested that the periodicity of the alternating α/β tubulin heterodimer is important not only for MT polarity (3) but also, to shape the energy landscape of the MT tail-coated surface.

The main finding from the maps presented here is that diffusion both along and across protofilaments requires the protein to cross an energetic barrier with size that is highly dependent on the precise properties of the tubulin tails of the MT matrix on which the protein diffuses.

**Energy Barrier for Protein Diffusion.** To quantitatively calculate the energy barrier for protein diffusion, we chose to break down the MT system into two components—body and tails—and to calculate the energy barrier for each component separately. We, therefore, constructed two types of systems: 1) systems in which diffusion is governed by the MT tails in that one or both of them remain charged, whereas the body residues are neutralized as schematized in the cartoon diagrams in Fig. 4 A, C, and D and 2) a system in which diffusion is governed by the MT body in that it remains charged, whereas charged tail residues are neutralized as schematized in the cartoon diagrams in Fig. 4B.

For each of the systems with charged tails (Fig. 4 A, C, and D), we created several subsystems (Methods has details) in which we successively neutralized the charges of the tail residues from 1)
The energy barrier is defined as diffusion along and across protofilaments. For each system, the potential is one-dimensional (as can be appreciated from Fig. 4). The preexponent will be referred to herein as $D_0$. Following Zwanzig’s derivation, $\gamma$ is proportional to $\exp(\varepsilon/K_BT)$, where $\varepsilon$ is the ruggedness of the potential energy (Fig. 4, Top) (42). Accordingly,

$$
D = D_0\varepsilon^{\frac{\sigma_{\text{tail}}}{K_B T}} \exp\left(\frac{\Delta G}{K_B T}\right),
$$

where $\varepsilon$ is the step size between the periodic wells, $\sigma_0$ is the curvature of the energy minima, $\sigma_b$ is the curvature at the top of the energy barrier, $\gamma$ is the friction, $K_B$ is the Boltzmann constant, and $T$ is the temperature (41). The preexponent will be referred to herein as $D_0$. Following Zwanzig’s derivation, $\gamma$ is proportional to $\exp(\varepsilon/K_BT)$, where $\varepsilon$ is the ruggedness of the potential energy (Fig. 4, Top) (42). Accordingly,

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or the tubulin tails ($E_{prot\text{-}tail}$) (Fig. 5A and B). It is clear from the heat map (Fig. 5A) that the tail-bound fraction (bottom right side of Fig. 5A) is more populated than the body-bound fraction (top left side of Fig. 5A). Based on the heat maps, we calculated the fraction of time in which the protein was bound to the MT body or to the tails (Methods has details) as a function of charge density for each system. When increasing the charge density of the tails, the protein shifted gradually from the body-bound mode (Fig. 5C, black) to the tail-bound mode (Fig. 5C, white). In the WT system in which the tails have their native charge, the protein is almost solely in the tail-bound mode. Hence, we can conclude that protein diffusion along WT MT is mediated mostly by the MT tails.

To further validate our observations from the CG simulations, we performed five repeats of all-atom simulations lasting 500 ns. In each simulation, we varied the initial position of the diffusing protein relative to the MT. In the all-atom simulations, we also found that the protein was mostly bound to the tails as can be seen from the heat map in Fig. 5B and in the selected conformations from the all-atom simulations (Fig. 5D and Movie S5).

### Effect of Posttranslational Modifications on Protein Diffusion

Having found that tubulin tails are the main effectors of protein diffusion along MTs, we sought to investigate the effect that PTMs have on protein diffusion. Two common PTMs are polyE and polyG, which arise from the addition of polyglutamate or polylglycine chains, respectively, to the $\gamma$ carbon of one of the tubulin tails. Based on the different molecular properties of polyG and polyE chains, it is expected that they will exert different effects on protein diffusion along MTs. The results that we obtained thus far suggest that increasing tail charge may slow down protein diffusion. Therefore, we would expect that the addition of highly charged polyE chains to the MT tails will slow protein diffusion on MTs, while the addition of neutral polyG may not affect diffusion.

To examine the effect that PTMs have on protein diffusion, we constructed systems with polyE/G chains of various lengths branching off both $\alpha$ and $\beta$ tubulin tails. The size of the polyE/G branches ranged from 5 to 20 additional E/G residues per tubulin tail (Methods has details; see Movies S2 and S4 for representative simulations). We found that the addition of polyE or polyG decreases the longitudinal diffusion coefficient $D_y$ (Fig. 6A, black and white bars, respectively) and that the effect is significantly higher on addition of polyE. The origin of this effect may be that the addition of polyE but not polyG also leads to an increase in the interaction energy between the diffusing protein and the tubulin tails (Fig. 6B and C; illustrations of protein–tail interactions are shown in Fig. 6D–F). Interestingly, although addition of polyG leads to decrease in $D_y$, it leads to increase in the lateral diffusion coefficient $D_x$ (Fig. 6A, Inset, white bars). A plausible explanation for this dual effect exerted by additional glycine chains on diffusion is that, on one hand, polyG acts to screen electrostatic interactions between the protein and charged tail residues and that, on the other hand, the chain has a crowding effect. The former is expected to facilitate diffusion, but the latter is expected to slow it down. Accordingly, the increase in lateral diffusion coefficients due to addition of polyG is in concert with our observation that systems with neutral $\alpha$ or $\beta$ tails have low-energy barriers for side stepping (Figs. 3B and C and 4C and D), which supports the possibility that polyG screens electrostatic interactions.

### Conclusions

Tubulin tails are known to regulate MT function and interactions (5–10). Computational and experimental studies have revealed molecular properties of tubulin tails (11–13), and there is accumulating evidence that PTMs of tubulin tails are abundant and crucial for proper MT function (17, 18). However, due to their...
intrinsic disorder, the molecular details of the regulatory role that tubulin tails and their modifications have on MT interactions with other proteins remain poorly understood (19, 20).

In this study, we used CG and atomistic MD simulations to unravel the molecular details of the role that tubulin tails and their modifications have on the protein diffusion on MT—a transportation mode used by many proteins (35). We found that increasing salt concentration and removal of tubulin tails lead to faster diffusion of EB1, PRC1, and Tau on MTs. These results are in line with experimental observations (22, 27) and indicate that electrostatic interactions play a central role in diffusion of proteins on MTs. Notably, electrostatic interactions are also important in the diffusion of proteins along DNA (36, 43). Still, the effect that removal of tubulin tails has on the diffusion of two members of the kinesin family is ambiguous (25, 37), suggesting that the effect that tubulin tails have on diffusing protein may depend on the interacting protein.

Protein diffusion on MTs occurs in discrete steps in the size of a tubulin dimer (~8 nm) in accordance with the step size of 8 nm reported experimentally for EB1 (22), PRC1 (22), and human (44) and yeast kinesin 8 (37). The precise properties of the tails are directly linked to step size, directionality, and energy barriers of proteins diffusing on MTs. Specifically, removal of tubulin tails changes step size to ~4 nm (tubulin monomer) in contrast to what was suggested experimentally for various proteins diffusing on WT MT. A step size of 4 nm for nonmotor MAPs was not previously reported and is expected for tails with fewer charges, but it may also be protein dependent.

We also found that diffusion occurs both along and across (side stepping) protofilaments. Interestingly, it was shown recently that kinesin 8 uses side stepping in order to bypass obstacles (32, 33), such as other motor or nonmotor proteins. Hence, it is possible that side stepping is a general mechanism that proteins can use in order to bypass obstacles on MTs. The spontaneous diffusion of the three studied proteins on MT is bidirectional; however, on force application, they may have directional preference due to an asymmetric friction (22).

The energy barrier for protein diffusion on MTs was previously reported to be in the range of ~2 to 13 $K_BT$ (37, 45). However, the contributions of tubulin tails to the energy barrier and the energy barrier for side stepping were not yet determined. Here, we report that the energy barrier for tubulin tail-mediated diffusion is higher than the barrier of MT body-mediated diffusion and that the tails of β tubulin are the main contributor for this barrier. Finally, our findings provide biophysical insight to the still unresolved tubulin code. We found that PTMs can regulate the delicate balance between the affinity of proteins to MTs and the speed of diffusion, with polyE leading to a slowdown in diffusion of proteins on MTs and polyglycilation increasing the diffusion rate across MT protofilaments. This molecular perspective may have further implications on additional roles played by the tails modifications, including effect on MT mechanical stability and interactions of MTs with severing enzymes and motor proteins.

**Methods**

To study the diffusion of proteins along MTs, we constructed an MT lattice consisting of four protofilaments each consisting of six tubulin monomers. The coordinates of the MT lattice were based on the structure of a single isoform neuronal human MT (Protein Data Bank [PDB] ID code 5C0J) (46). The sequences of disordered tails follow the tails of isoform α1A and β3, which comprise 13 and 24 residues, respectively, and they were added as linear chains to the C terminus of each tubulin monomer. The diffusing proteins used in this study are MT-binding domains of EB1, PRC1, and Tau.

The dynamics of protein diffusion along MTs was studied using CG MD simulations, and the interactions between the diffusing proteins and the MT are modeled by electrostatic interactions. The beads of the structured part of the MT (referred to herein as “MT body”) were kept fixed in our simulations, but the tails were flexible.

To study the contributions to protein diffusion along MT that arise from the electrostatic potential of the tubulin folded domains, the tails, and cross-talks between them, we constructed several variants of MT where the charges of the body or of the tail residues were modified (Supplementary Material). The effect of polyE or polyG on protein diffusion along MT was studied by modifying the tail through the addition of polyE or polyG chains, respectively. The polyE/G chains were added by creating a peptide bond between the amino terminal of the chains and the Cα atom of glutamate 445 in α tubulin or glutamate 435 in β tubulin. These sites were previously identified as common positions for polyE (47-49). We constructed CG systems with 5, 10, 15, and 20-residue polyE/G chains branched off the α and β tails.

To complement the CG model, we also performed all-atom simulations, which were performed on a smaller “slice” of MT consisting of three protofilaments, each consisting of four tubulin monomers. The atomistic simulations were performed for the MT lattice in order to quantify the conformational dynamics of the tails. In addition, simulations were performed on MT with EB1 to examine the interactions between EB1 and the MT body and disordered tails. Additional details can be found in Supplementary Material, Supplementary Methods.

**Data Availability Statement.** All data discussed in the paper have been deposited in Open Science Framework (https://osf.io/mz8yj/).

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