Analysis of the nucleotide-dependent conformations of kinesin-1 in the hydrolysis cycle

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Kinesin-1 motion on a microtubule (MT) is still receiving a great attention due to its relevance in understanding molecular motion triggered by adenosine triphosphate (ATP) hydrolysis. Recent experimental data on kinesin-tubulin-nucleotide interactions have clarified some of the conformational details involved in the hydrolysis process [T. Mori *et al.*, Nature (London) **450**, 750 (2007)]. Specifically, fluorescence resonance energy transfer was used to measure the affinity of motor domains to tubulin heterodimers. Our work is directly devoted to understand and reproduce the main output of these experiments as well as to go beyond and give a global dynamical picture of the whole hydrolysis cycle. We predict that phosphate groups have the ability to confine to the tubulin domains in order to explain the delay between ATP hydrolysis and head detaching, which seems crucial for the achievement of processivity. In our approach me make use of chemical kinetics complemented with stochastic molecular simulations of the elements involved.
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I. INTRODUCTION

In order to understand how kinesin walks along microtubules it is essential to clarify how each of the heavy chain domains interacts with tubulin heterodimers depending on the nucleotide state of the enzyme. Very recently[1,2] new sets of experiments have been performed with kinesin-1 in order to shed some light on this interaction. Even though these references do not exactly agree with each other concerning the relative position of the tethered head with respect to the attached heavy domain, they both give some pieces to complete the adenosine triphosphate (ATP)-head-tubulin puzzle. Our previous work on the electrostatic interactions on kinesin-microtubule systems[3] dealt with medium range forces that are based on Coulomb potentials and Debye screening effects. However, the nucleotide-dependent charge of each head was not modeled. The so-called question of how the chemical energy is converted into mechanical work was reduced to the question of how the hydrolyzed nucleotide manages to carry with itself the head domain when repelled from the microtubule. Furthermore, even though the role of the γ-phosphate group was known to be crucial in the process of head detaching,[4] it was not clear how P_i could have such a regulating task. In this section we will give some new insight on the mechanochemical energy conversion clarifying how the role of the phosphate group is essential to the whole cycle.

In Ref. 1 some stationary situations between the head domains and the microtubule have been characterized with fluorescence resonance electron transfer (FRET) technique. The main scenarios can be summarized as follows. In the presence of no nucleotide, both heads of the dimer are mainly attached to the microtubule. If we add AMP-PNP (5′-adenylyl-beta, gamma-imidodiphosphate), a nonhydrolyzable analog of ATP, we can see a similar situation, i.e., both heads are attached, but the FRET distributions are less broad, i.e., the bindings are more tight. If we prepare a system where only ADP (Adenosine diphosphate) is available, then there are two mainly stable configurations: The heads that are attached to tubulin have no nucleotide in the pocket, while heads that are tethered but relatively far away from the microtubule have ADP in their pockets. Furthermore, if we add P_i in a quantity that $[P_i]/[\mathrm{ADP}] \sim 5 \times 10^4$ we can still see how the motor is mainly attached to a single head but a two-bound-state appears with a relatively small frequency.

Given this experimental information we will now try to complete our previous analysis of kinesin motion by modeling the interaction between four objects: A tubulin dimer (T), a kinesin's head (H) domain, an ADP, and a $P_i(P)$ group. Our main goal will be to describe the experimentally observed states of T-H as a function of the different nucleotide states. We will introduce effective interactions between these four elements shedding some light on the question of how the stored energy in ATP is able to produce the necessary head detachment. In fact, it will be shown that the role of the phosphate P_i is pivotal in understanding the whole mechanochemical cycle of kinesin. Specifically, it is the ability of P_i to confine itself near negatively charged structures such as ADP or tubulin dimers at small distances what allows ATP energy storage and delayed head detachment.

On the other hand, the other main assumption is based on the catalytic activities of the enzyme. It is commonly accepted that an enzyme is able to lower the activation barrier of the ATP allowing the hydrolysis to occur with a much

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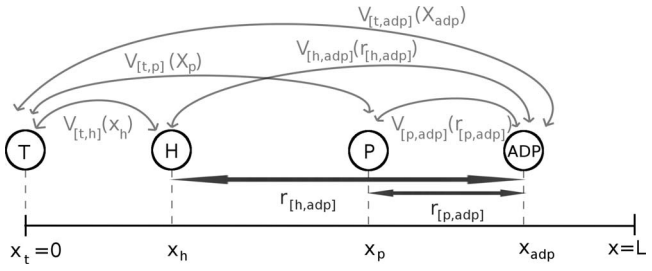

FIG. 1. Scheme of the four elements of the model: tubulin (T), kinesin head (H), ADP, and P. We show how they move in a 1D system bound between $x=0$ and $x=L$. We explicitly write the interaction potentials with their position or distance dependences.

higher rate. We will incorporate this effect in our modeling in a more detailed way. Specifically, we consider that the activation barrier, given by a Gaussian potential term, is transferred from the ADP-P interaction to the interaction between the ADP and the head domain. In other words, when the ATP binds the pocket, the phosphate group and the ADP will not be strongly confined anymore and thus after some fluctuations they will repel each other, while the confinement is transferred to the head, so the ADP is trapped in the head domain. In other words, the activation barrier of the ATP molecule is converted into a closing of the pocket. Additionally, we will consider that such a barrier transference produces the opposite effect on the other-tethered head, which causes the opening of the pocket and the subsequent ADP release.

First we will model the interactions between the four objects of the system. Then we will show the results, beginning by the simulation of the situations that have been performed in Ref. 1, and finally extrapolating the model to an ATP hydrolysis, which is a situation that is not trivial to measure in an experiment.

II. MODELING THE INTERACTIONS

In this section we will introduce the analytical expression for effective potentials and subsequent forces between the six pairs formed by T, H, ADP, and P, as shown in Fig. 1. Most of them are essentially Coulomb potentials with a screening exponential correction due to the ionic environment. However, there are other interactions that even though we have tried to write them in the simplest form, there are no available standard descriptions of them, as far as we know. The main goal is to describe the phenomenology involved in nucleotide-dependent kinesin-MT interactions, but some of the specific quantities presented here, even if they are reasonable, are introduced without experimental support. Specifically, it is the confinement effect of ADP into H, P into ADP, and P into T that is not included in the Coulomb–Debye terms, so then we will make use of Gaussian terms in order to model the confinement barriers and wells. Although all the details of the mechanochemical cycle model will not be completely described until last section (III B) we will anticipate the scheme of the whole system in Fig. 2.

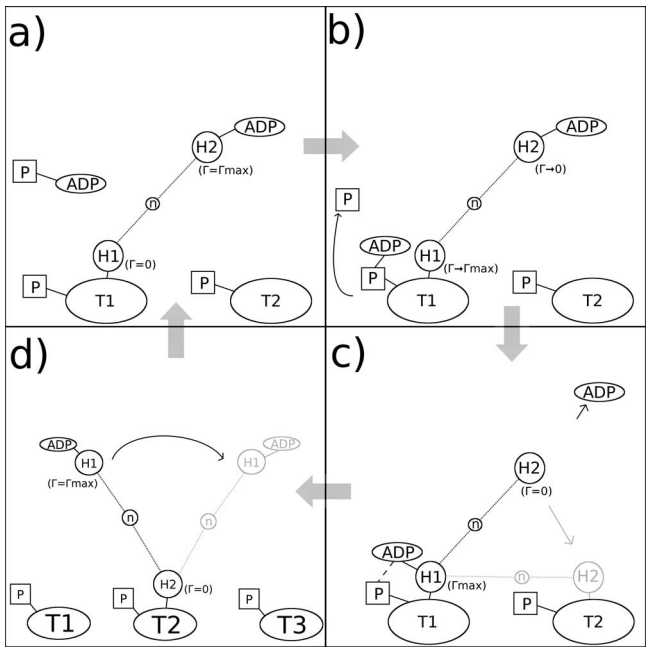

FIG. 2. Scheme of a complete ATP hydrolysis cycle in a kinesin-1. In (a) we can see how kinesin is on its parked state, trailing head H1 attached to the tubulin site T1 while leading head H2 is in a tilted position toward the plus end of the protofilament. Both heads are linked by the neck. In the scheme we also draw a second tubulin site T2 where kinesin is going to walk to. Both tubulin sites are sketched with bound phosphate groups. In this parked configuration, the enzymatic state of H1 corresponds to $\Gamma=0$, while H2 has $\Gamma=\Gamma_{max}$. This is the state where kinesin spends most of its time. However, an ATP molecule will eventually diffuse to the trailing head domain, as is the case of the picture. The ATP binding corresponds to the transition to the next part of the figure. In (b) the ATP molecule is bound to the tubulin site T1 through the phosphate group, while the head H1 and the ADP attract each other. Upon the enzymatic activity of H1, the state of H1 changes to Γ_{max} and the enzyme H2 commutes to $\Gamma=0$. In (c) we see the effects of the hydrolysis. The phosphate group and ADP are still bound but with a much lower protection barrier, since most of this confinement energy has been transferred to the ADP-H1 complex. On the leading head domain, ADP has eventually left the enzymatic pocket due to the $\Gamma=0$ state of the enzyme. This implies a change in the charge sign in H2 and thus the leading head collapses to the next tubulin site. Why the collapse is directed toward the plus end of the microtubule and not to the minus end is not to be understood in terms of the tilting of the parked state but to the interaction of the neck charge with the tubulin dipole moment, as shown in Fig. 3. Finally, in (d) we observe the eventual transition that occurs upon ADP-P breaking. In our previous work we have already seen how the competition between this breaking process and the collapse time of the leading head allows to understand the existence of processivity in kinesin motion.

A. Tubulin-head (*T-H*) interaction

The interaction of a tubulin dimer with a kinesin's head is modeled by considering that the tubulin T is simply a charge Q_t with an excluding volume radius R_t. It interacts electrostatically with the head, which is also modeled as a charge Q_h with radius R_h. We know that a tubulin subunit can have about -27 electronic charges[5,6] or $-e$.[7] We use the latter value. For the kinesin head we will use $Q_h=0.5$. The reason to consider the head as an effective positively charged structure is to be able to explain the affinity of free heads for the microtubule. The specific value of $+1/2e$ is based on the fact that we want a MgADP, which has $-e$, to be able to invert the sign of the head. So, if we choose these values, a head with MgADP (we will omit the Mg for simplicity) will have a charge of $-1/2e$ and with ATP the total charge will be $-3/2e$.

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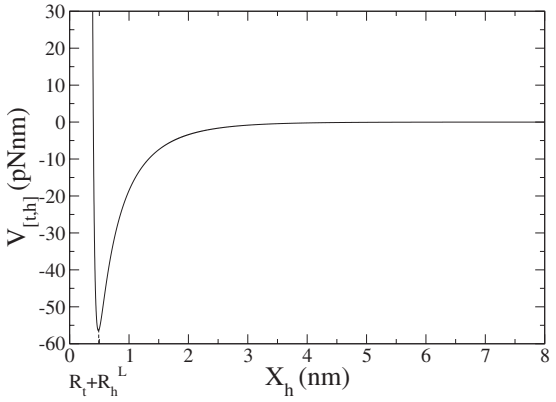

FIG. 3. Potential between the head and the tubulin along a 1D coordinate system. We can see how the head is mainly attracted to the microtubule due to its positive charge, even though it cannot penetrate it beyond R_t+R_h. In this potential, Debye screening predominates at long distances, while van der Waals repulsion dominates at short distances.

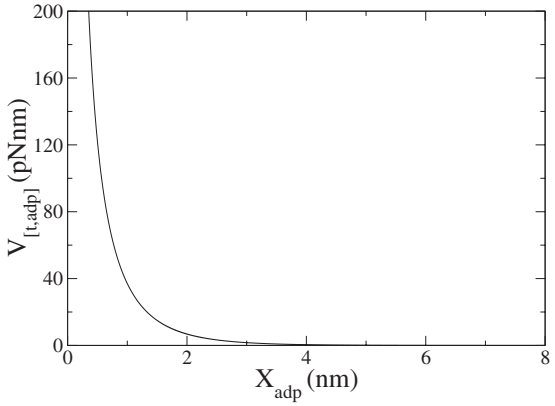

FIG. 4. Interaction potential between the ADP molecule and the microtubule along the coordinate that defines the position of ADP, x_{ADP}. Such an interaction is always repulsive, but the existence of a reflecting barrier at $x_{\mathrm{ADP}}=L$ due to a finite ADP concentration allows the ADP to eventually approach the tubulin helped by thermal fluctuations.

The Coulomb interaction with a screening correction can be written as

$$V(r_{ij}) = \frac{kQ_iQj}{\epsilon_r r} e^{-r_{ij}/\lambda_D}, \tag{1}$$

where $k \simeq 230$ pN nm^2 e^2, $\epsilon_r \simeq 80$, and $\lambda_D \sim 1$ nm are $1/(4\pi\epsilon_0)$, the relative permittivity, and the Debye length, respectively (all in [nm-pN-s] unit system). The only missing ingredient is a contact repulsion in order not to allow H to penetrate T. This repulsion is incorporated through a van der Waals term inversely proportional to x_h^{12}. We write x_h for the position of the head, which is bound between the interval $[0,L]$ and $L=8$ nm. We fix the position of the external surface of the microtubule dimer at $x_t=0$ and we suppose its motion is negligible compared to the motion of the head. Then, we can write for the whole potential

$$V_{[t,h]}(x_h) = \frac{kQ_tQ_h}{\epsilon_r} e^{-x_h/\lambda_D}\left(\frac{1}{x_h} - \frac{(R_t+R_h)^{11}}{12x_h^{12}}\right). \tag{2}$$

This potential accomplishes the fact that the head has significant affinity for the microtubule when free from any nucleotide. We can see the plot of the potential in Fig. 3.

B. Tubulin-ADP (*T*-ADP) interaction

The ADP molecule is again a charged object but now with charge $Q_{\mathrm{ADP}}=-1e$ and radius R_{ADP}. Except from these two differences, the T-ADP potential is the same as the T-H potential. But now the ADP, being negative, will be repelled away from the microtubule and only thermal noise will be able to force an approach. The ADP particle, as all of them except the tubulin, can swim along the one-dimensional (1D) path between $x_{\mathrm{ADP}}=0$ and $x_{\mathrm{ADP}}=L$. Unlike other works in this field, where x is used to denote the direction of motion along the microtubule axis, here x is the radial height away from the microtubule, perpendicular to the filament axis. At $x_{\mathrm{ADP}}=L$ there is a reflecting barrier for the ADP as well as for P and H. In the P and ADP cases, such a barrier is a way to introduce the concentration of these molecules. Specifically, the closer the barrier to the microtubule, the larger the effective concentration. The existence of a reflecting barrier for H is simply a way to consider the tethering of the head by the rest of kinesin structure. Since in this model we are not considering dimeric-structural properties, we do not want the head to diffuse more than ~ 8 nm away from the microtubule. Thus we can write

$$V_{[t,\mathrm{ADP}]}(x_{\mathrm{ADP}}) = \frac{kQ_tQ_{\mathrm{ADP}}}{\epsilon_r} e^{-x_{\mathrm{ADP}}/\lambda_D}\left(\frac{1}{x_{\mathrm{ADP}}} + \frac{(R_t+R_{\mathrm{ADP}})^{11}}{12x_{\mathrm{ADP}}^{12}}\right). \tag{3}$$

With such a potential, as can be seen in Fig. 4, only thermal fluctuations allow the ADP to explore the vicinity of tubulin.

C. Tubulin-phosphate (*T*-*P*) interaction

We know that when two charges are sufficiently close, other effects apart from the Coulomb interaction appear. In fact, all the chemical types of bonding are based on this highly nontrivial electrodynamics. The structure of the effective potential between the microtubule and the phosphate group is more subtle than the T-ADP potential. At medium and long distances, we can consider the same Coulomb–Debye potential as in the two previous cases. However, when P will explore due to thermal noise, the region close to the tubulin, it will be able to bind it due to a well in the potential. With such a profile, the phosphate can eventually bind the microtubule in a higher energy configuration for a reasonably long time. We model this confinement effect through the addition of a Gaussian potential to the Coulomb–Debye profile. The Gaussian is centered at $x_p=R_p$, has a height $E_{[t,p]}$ and a width σ, such that $1/2\sigma=A_{[t,p]}$. We can write the potential as

$$V_{[t,p]}(x_p) = \frac{kQ_tQ_p}{\epsilon_r x_p} e^{-x_p/\lambda_D} - E_{[t,p]} e^{-A_{[t,p]}(x_p - R_p)^2}. \tag{4}$$

This potential plotted in Fig. 5, even though is partially phenomenological, achieves the interesting property that allows the P_i confinement. Such a confinement is a hypothesis that is part of our model, but it allows to understand the stability of the AMP-PNP states of kinesin when attached to the microtubule and also prevents the ATP from being repelled

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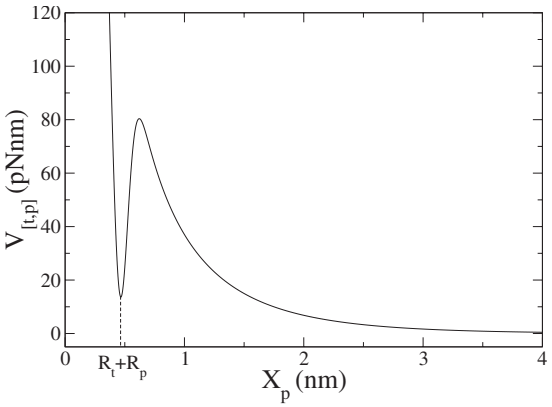

FIG. 5. Potential between the phosphate P_i and the microtubule T as a function of P position, x_p. We can notice how this potential is very similar to $V_{[t,\mathrm{ADP}]}$ except at very short distances (R_t+R_p), where a confinement phenomenon appears. Our hypothesis is that the phosphate group is able to bind the microtubule. This hypothesis can explain the affinity of AMP-PNP state of kinesin to the microtubule and also the appearance of a low but finite frequency state of kinesin with two heads bound in the presence of free ADP and a big concentration of P (Ref. 1). Such a hypothesis suggests that some tubulin sites at high $[P]$ may be occupied by a phosphate group.

from the microtubule before the hydrolysis process. Furthermore, the appearance of a relatively low-frequent state of two-bound-head kinesin in ADP solution with high $[P]$ can also be explained with the confinement of P in T. However, such a hypothesis implies the fact that some tubulin sites (at high $[P]$) may be occupied by a phosphate group. This phenomenon could be tested experimentally by labeling the phosphates with isotopic techniques, and maybe it is this effect that determines the preference of kinesin to walk above the beta subunits of the protofilament.

D. Head-phosphate (*H-P*) interaction

We will simply consider that the phosphate group does not interact with the head. At first sight, it may seem counterintuitive, but it is perfectly reasonable. There is a strong connection between the head and P, but this connection has the ADP molecule as a mediator. Thus we write

$$V_{[h,p]}=0 \quad F_{[h,p]}=0. \tag{5}$$

We could consider simple charge-charge interaction between the phosphate group and the head, but the obtained results would be essentially the same.

E. Head-ADP (*H*-ADP) and phosphate-ADP (*P*-ADP) interactions: Enzymatic barrier transfer and coordination between the two heads

The interactions of ADP with H and P constitute the main core of the model concerning enzymatic activity. It is known that enzymes are able to reduce the height of the activation barrier of the substrate facilitating the formation of the product. As we are modeling the interaction between ADP and P we need to consider such an effect. But there are evidence[4] that kinesin not only lowers the ATP activation barrier but also is able to close the nucleotide pocket upon ATP hydrolysis. Thus it is reasonable to suppose that the activation barrier is transferred from the ADP·P complex to the ADP·H system. We model this barrier term as

$$E_A = E_\Gamma e^{-E_\Gamma (r-R_\Gamma)^2}, \tag{6}$$

where the distance r can be either $r_{[h,\mathrm{ADP}]}$ or $r_{[p,\mathrm{ADP}]}$, depending on whether the enzyme is in a state $\Gamma=1$ or $\Gamma=0$. In fact, the $\Gamma=0$ state corresponds to the ATP molecule in a stable conformation and the nucleotide pocket in an open configuration, while $\Gamma=1$ corresponds to an unstable ATP and a confined H-ADP state (the ADP trapped in the closed pocket). We can write

$$V_{[h,\mathrm{ADP}]}(r_{[h,\mathrm{ADP}]}) = \Gamma E_\Gamma e^{-a_\Gamma (r_{[h,\mathrm{ADP}]}-R_\Gamma)^2} + \frac{kQ_hQ_{\mathrm{ADP}}}{\epsilon_r} e^{-r_{[h,\mathrm{ADP}]}/\lambda_D} \times \left(\frac{1}{r_{[h,\mathrm{ADP}]}} - \frac{(R_h+R_{\mathrm{ADP}})^{11}}{12 r_{[h,\mathrm{ADP}]}^{12}} \right) \tag{7}$$

and

$$V_{[p,\mathrm{ADP}]}(r_{[p,\mathrm{ADP}]}) = (1-\Gamma) E_\Gamma e^{-A_\Gamma (r_{[p,\mathrm{ADP}]}-R_\Gamma)^2} + \frac{kQ_pQ_{\mathrm{ADP}} r_{[p,\mathrm{ADP}]}}{\epsilon_r} e^{-r_{[p,\mathrm{ADP}]}/\lambda_D}, \tag{8}$$

where we have added to each case the Coulomb–Debye contribution with short-range repulsion in the H-ADP case.

In this context, the activity of the enzyme is to change the value of Γ, which we will consider as an instantaneous switch between $\Gamma \in [0, \Gamma_{\max}]$. We will discuss later which value of $\Gamma_{\max}$ is more appropriate in order to agree with the experimental data. Furthermore, the Γ parameter is involved in the coordination between the two heads of kinesin. What is accepted is that ATP binding at the attached head promotes ADP release on the tethered head,[8] but it is not known how both heads communicate, even though some mechanisms are proposed. First, mechanical strain models in which ATP binds on the attached head induce a strain in the other head. Reference 9 gives a model where both heads are connected by an elastic interaction that coordinates the torsional state of both heavy chains. This interaction is complemented with an energy barrier that accounts for an ATP-dependent state of the mutual states. On the other hand, extra electrostatic repulsion due to the presence of ATP in the attached head may help ADP release,[10] but it is not clear yet how this interaction can be channeled along 8 nm without being completely screened. We will consider here the following assumption. Let Γ_1, Γ_2 be the states in heads 1 and 2, respectively. Then, the states must always hold $\Gamma_1+\Gamma_2=2-\Gamma_{\max}$, which implies that if one of the heads changes its state the other head changes it automatically. Such an entanglement of unknown origin to us allows the two-head coordination, which is necessary for a processive hand-over-hand motion. The role of Γ is quite similar to the commutators of brushed dc electric motors.

In Fig. 6 we can see plots of $V_{[p,\mathrm{ADP}]}$ and $V_{[h,\mathrm{ADP}]}$ at both $\Gamma=0$ (solid line) and $\Gamma=\Gamma_{\max}$ (dashed line). We can explicitly see the transfer of the activation barrier from $V_{[p,\mathrm{ADP}]}$ to $V_{[h,\mathrm{ADP}]}$ when $\Gamma: 0 \rightarrow \Gamma_{\max}$ and from $V_{[h,\mathrm{ADP}]}$ to $V_{[p,\mathrm{ADP}]}$ when

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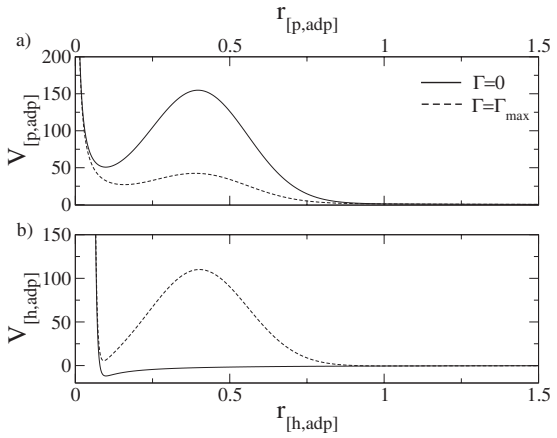

FIG. 6. Interaction potentials of ADP with P (upper figure) and with H (lower figure). Each potential is plotted for $\Gamma=0$ (solid line) and $\Gamma=\Gamma_{\max}$ (dashed line). (a) $V_{[p,\mathrm{ADP}]}$ vs $r_{[p,\mathrm{ADP}]}$, which is the distance between P and ADP. We can see how P and ADP can be confined at close distances (≤ 0.25 nm) at an energy of ~ 50 pN nm, which is the enthalpic value for the ATP hydrolysis. When they are mutually confined at this state we can say that we have an ATP molecule. Nevertheless, we can see that when $\Gamma=\Gamma_{\max}$, which here is 0.75, the protection barrier is strongly reduced, allowing a fast hydrolysis inside the enzymatic cavity. (b) $V_{[h,\mathrm{ADP}]}$ vs $r_{[h,\mathrm{ADP}]}$. When $\Gamma=0$ the head and the ADP are electrostatically attracted, and ADP can bind the catalytic pocket even though the average time of residence inside this cavity is low, especially if the head is bound to the microtubule. However, when $\Gamma=\Gamma_{\max}$ the protection barrier is transferred from $V_{[p,\mathrm{ADP}]}$ to $V_{[h,\mathrm{ADP}]}$, and then after ATP hydrolysis the ADP remains inside the pocket until Γ returns to a zero value promoting ADP release.

$\Gamma:\Gamma_{\max}\rightarrow 0$. In our model we are considering that the height of the protection barrier is ~ 150 pN nm, but as it represents the activation barrier of an ATP molecule it should be much higher. We are considering here a lower value in order not to make the computational time unavailable. Such a reduction does not significantly change the scenario in a qualitative way. However, a more realistic situation would require a much higher barrier in order to obtain an $\sim$1-week-stable ATP.[11] The mechanism of the barrier transfer is the following. When the ATP enters into the kinesin pocket, which in fact means that the ADP enters into the pocket and what is more important, the phosphate group is confined to the microtubule, the barrier is transferred to the interaction between the head and the ADP. Then, the repulsion between P and ADP without a high protection barrier helps the ADP expulsion away from the microtubule, but now as the head is confined to ADP, both ADP and H are expelled from the microtubule surface, which is the rising process of the trailing head described in Ref. 3. When this head reaches the parked state and an ATP binds the attached head and it changes it barrier-Γ state, the tethered head changes its Γ-state as well in the reverse order and then ADP is easily released from the pocket, which causes the head to be attracted again by the microtubule, but this time to the next tubulin site. It is in Ref. 3 where the role of the neck in producing directional motion is discussed, while here we are focusing on how the substeps of the whole cycle are sequenced as the nucleotide states change in each of the heads.

Furthermore, this approach allows us to model the experimental situation where we substitute ATP with the nonhydrolyzable analog AMP-PNP. We will consider that the AMP-PNP simply does not activate the switch in Γ values, so we will see how the head domain remains stably bound to the microtubule, thanks to the confinement of the phosphate, which in AMP-PNP is supposed to be able to bind the microtubule as in the ATP case. Numerical details and parameters of the model are shown in Table I.

TABLE I. Parameters of the model with their values and units.

Parameter	Value	Units
ϵ_r	80	Dimensionless
λ_D	1	nm
k	230	pN nm^2 e^2
$Q_p=Q_{\mathrm{ADP}}$	-1	e
Q_t	-35	e
Q_h	$-1/2$	e
L	8	nm
k_BT	4.1	pN nm
Δt	$\lambda_p 10^{-7}$	s
R_p^{Stokes}	0.05	nm
$R_{\mathrm{ADP}}^{\mathrm{Stokes}}$	0.20	nm
R_h^{Stokes}	2.00	nm
R_t	0.10	nm
R_{ADP}	0.10	nm
R_H^L	0.40	nm
R_H^R	0.00	nm
R_p	0.45	nm
R_Γ	0.40	nm
$A_{[t,p]}$	100	nm^{-2}
A_Γ	20	nm^{-2}
$E_{[t,p]}$	125	pN nm
E_Γ	150	pN nm

III. NUMERICAL RESULTS AND DISCUSSION

We perform Langevin simulations in one dimension with a Gaussian white noise of intensity $k_BT=4.1$ pN nm, where the drag coefficients follow the Stokes relation $\lambda=6\pi R\eta$, where η is the viscosity of the medium, which we approximate to be equal to the viscosity of the water, $\eta_{\mathrm{H_2O}}=10^{-9}$ pN s nm^2. Here R is the Stokes radius, also called van der Waals radius, and it is different from each of the four objects. Specifically, we consider that it is infinite (very large compared to the rest) for the microtubule, while $R_p^{\mathrm{Stokes}}=0.05$ nm, $R_{\mathrm{ADP}}^{\mathrm{Stokes}}=0.2$ nm, and $R_h^{\mathrm{Stokes}}=2$ nm. The equations are the following:

$$\lambda_p\dot{x}_p = F_{[\mathrm{total},p]} + \xi_p(t), \tag{9}$$

$$\lambda_{\mathrm{ADP}}\dot{x}_{\mathrm{ADP}} = F_{[\mathrm{total,ADP}]} + \xi_{\mathrm{ADP}}(t), \tag{10}$$

and

$$\lambda_h\dot{x}_h = F_{[\mathrm{total},h]} + \xi_h(t), \tag{11}$$

where

$$F_{[\mathrm{total},p]} = F_{[t,p]} - F_{[p,h]} - F_{[p,\mathrm{ADP}]}, \tag{12}$$

$$F_{[\mathrm{total,ADP}]} = F_{[t,\mathrm{ADP}]} + F_{[\mathrm{ADP},h]} + F_{[p,\mathrm{ADP}]}, \tag{13}$$

and

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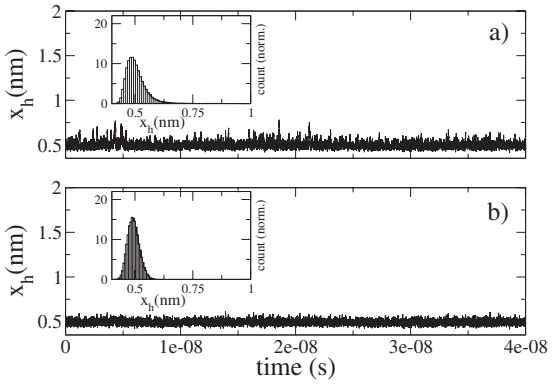

FIG. 7. Trajectories and normalized histograms for x_h (a) with no nucleotide and (b) with AMP-PNP added. We simulate the trajectories along $\sim 5 \times 10^{-8}$ s, which is enough for the histograms to be smooth. In (b), the initial conditions are taken with the AMP-PNP already bound to the *T*-*H* system. We can see how in the nucleotide-free case the distributions are broader, pointing to the fact that the head is not restricted by the confinement of a phosphate group to the microtubule, as is the situation in (b). With AMP-PNP added, we can observe transitions from AMP-PNP bound and AMP-PNP-free, but a high concentration of the nonhydrolyzable ATP analog (1 m*M* in Ref. 1) ensures that the head will mostly be in the state we show in the figure.

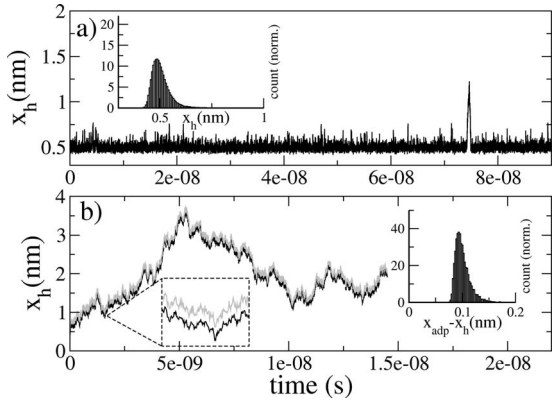

FIG. 8. (a) Two trajectories of the head and (b) the head and ADP vs time for (a) $\Gamma=0$ and (b) $\Gamma=0.75$. (a) This case, where $\Gamma=0$, is quite similar to the nucleotide-free solution, as the head is almost permanently collapsed to the microtubule. The normalized distribution is also very similar to the one observed in Fig. 7(a). In (b) we have $\Gamma=\Gamma_{\max}$, so then the head (black trajectory) is no more bound to the microtubule (located at $x=0$). In gray we explicitly plot the trajectory for the ADP in order to illustrate how the mutual confinement of ADP and *H* allows the head expulsion from the tubulin site. The left inset shows a magnification of these two trajectories. The right inset shows the distribution of $x_{\mathrm{ADP}}-x_h$, which is peaked at 0.1 nm. The distributions show no finite frequencies for distances bigger than 1 because there are no ADP release transitions during the simulation.

$$F_{[\mathrm{total},h]} = F_{[t,h]} - F_{[\mathrm{ADP},h]} + F_{[p,h]} \tag{14}$$

are the forces associated with the potentials by $F_i = -dV_i/dx_i$. Furthermore, we fix $x_t=0$.

A. The experimental cases

Now we will use the potentials described above to emulate the situations measured in Ref. 1. First, we will simulate only kinesin with tubulin. Later, we will "add" AMP-PNP and then we will study the system with ADP and finally with ADP+*P*.

1. Kinesin with no nucleotide and AMP-PNP solution

In order to reproduce the case where kinesin has no available nucleotide or P_i, we will fix the tubulin at $x=0$ and allow the head to move along $x_h \in [0,L]$ interacting with V_{th}. This case is quite simple, since the positively charged head likes to be near the negatively charged microtubule. When we add AMP-PNP, a nonhydrolyzable analog of ATP, it is observed that both heads of kinesin stably bind the microtubule. In the context of our approach this means that each head has an AMP-PNP attached with a phosphate group confined to the microtubule. Furthermore, as the AMP-PNP is not able to activate a switch in the Γ-state of the enzyme, the head remains attached to the tubulin. This case is quite interesting, since it is supposed to be a frozen image of what happens exactly after ATP binding, but without destabilizing the system toward a power-stroke cycle.

Thus these two cases, with no nucleotide and with AMP-PNP added, are quite similar and experiments confirm it. We show in Fig. 7 two trajectories of the head, one (a) with no nucleotide and the other (b) with AMP-PNP added. In both cases the head remains bound to the microtubule, but in the presence of AMP-PNP and due to *P* confinement, the distribution in the latter case is wider, as it is experimentally found in Ref. 1. There is relatively small portion of the x_h trajectory in the nucleotide-free case where the head is unbound from the microtubule, which corresponds to the experimentally observed one head bound state that appears with low frequency in the FRET setup of Ref. 1.

2. ADP

In the case where we add ADP to the motor-microtubule solution we can distinguish two situations, depending whether the head is in a Γ-state or in the other. If $\Gamma=0$, the head will not be able to trap an ADP in its interior and then it will collapse to the microtubule, while the ADP will mainly remain away from the heavy chain domain. Thus the situation can be summarized as ADP diffusing in the media and an empty head attached to the microtubule, as in the case where we add no ADP at all. However, if the head changes to a $\Gamma_{\max}$ state, it will have the ability to trap an ADP, so then the head and the ADP will diffuse together away from the microtubule. Such a diffusion is biased by the repulsion of the whole ADP-*H* system, which is negatively charged. Additionally, if we are supposing that the Γ-states of both heads in each dimer are entangled in such a way that in each head the state has to be different, we obtain a scenario where one head is nucleotide-free and attached to the microtubule while the other head is occupied by an ADP molecule and repelled by the tubulin electric field. Eventually, the ADP can overcome the protecting barrier and produce the collapse of the tethered head toward the microtubule again, and even though it is not the most probable situation, the FRET distributions observed in Ref. 1 that a two-bound-head state is also plausible (with low frequency). If the tethered head prefers to bind an ADP instead of being nucleotide-free we can guess that there is a confinement potential between the head and the ADP that makes the energy level of the *H*-ADP complex lower than the two elements alone. In Fig. 8 we show our results for this case.

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3. ADP+P

When adding P_i to the ADP solution the state where one head is bound and the other is tethered changes to a state where kinesin can have one or both heads attached, showing bimodal diagrams from FRET experiments.[1] In the context of our model the explanation is straightforward. When we add phosphate groups to the system (and the experiments add a $[P]=10$ mM for $[\mathrm{ADP}]=200$ nM), the tubulin sites may be mostly occupied by P. Kinesins are with one attached head and with another head unbound from tubulin. However, because $\Gamma_{\max}<1$, there is still some affinity of ADP for the phosphate, i.e., if we use $\Gamma_{\max}=0.75$ there is still a 1/4 fraction of the activation barrier that prevents ADP $\hat{A}|P$ binding (and confines them if the barrier is surmounted). Then, the ADP-tethered head fluctuates and eventually the ADP interacts with the microtubule-confined P. This results in a relatively short lived state where the head is bound to the microtubule via the chain T-P-ADP-H. In Ref. 1, the peaks of the bimodal distribution show that the peak corresponding to a one-head-bound state doubles the counting number of the two-head-bound peak. This indicates that the two-head-bound state is not so short lived in comparison with the one-head-bound state. On the one hand the former case is magnified by adding a big concentration of P, but on the other hand this suggests that the tethered head is quite close to a tubulin binding site when kinesin is on its parked state. This corresponds to a strongly tilted parked state, as it seems to indicate the results in Ref. 8 following the interpretation of Ref. 3. Under this interpretation, the interaction of tubulin dipole moments with the neck and with the tethered head charges produces a tilted parked state. The more tilted the dipole moment and the larger the charge of the neck linker, the more tilted the parked state. From the microsecond resolution trajectories measured in Ref. 8 we can estimate that the tethered head is less than 2 nm away from its target site. Then, in our simulations we have forbidden the head to diffuse beyond $x\sim 1.5$ nm. Furthermore, we have located the H-ADP complex confined to the T-P complex as initial conditions and we have let the dynamics evolve for several runs. With this methodology we are able to quickly see a good approximation of the frequencies in the two possible states. It is interesting how the appearance of the bimodal distribution due to the presence of a high concentration of P reveals the close distance between the tethered head and the microtubule, giving more support to our electrostatic model presented in Ref. 3. Furthermore, the low frequency of the state with both heads attached is in agreement with a configuration of elastic strain, as shown in Ref. 9.

We show in Fig. 9 the plots of three consecutive runs, all of them with confined-state initial conditions, for the position of the head. In the inset we show the normalized distribution of this position where two well distinguished peaks are observed with relative frequencies in good agreement with the data presented in Ref. 1. We can notice how we have restricted H motion into $x_h\in[0,1.5]$ nm in order to emulate the strongly tilted parked state of kinesin suggested by Refs. 2 and 3 among other works.

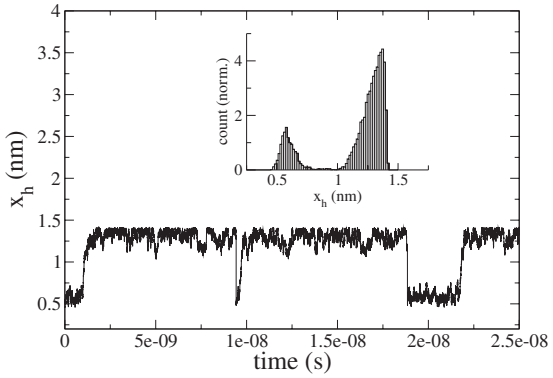

FIG. 9. Three different (and consecutive in time) runs for the trajectory of the head with $\Gamma=0.75$. The initial conditions locate the head confined due to the weak P-ADP interactions at high values of Γ. The bimodal distribution in the inset illustrates the existence of two metastable states, one where kinesin has one head bound and another where the dimer has its two heavy chains attached to the microtubule.

B. ATP hydrolysis scenario

Finally, we should discuss the case where we add ATP and hydrolysis occurs. Such a situation is not stationary as the others but dynamic, as it implies kinesin stepping. In fact, whenever we have a realistic solution, i.e., ATP with ADP and P, we can observe all the previous features in a sequential way. First, when kinesin is in its parked state, the dimer has one head bound to the microtubule with no nucleotide, while the other is tethered through the neck linker with an ADP bound. This is equivalent to the situation where we added only ADP. Later, when an ATP binds the attached-trailing head, the phosphate binds the microtubule, as in the AMP-PNP case. The difference is that AMP-PNP binds the tubulin with a long life time while ATP promotes the activation barrier transfer and it becomes hydrolyzed. But for a small time the AMP-PNP and the ATP scenarios are equivalent. After ATP hydrolysis kinesin returns to the parked state.

In our simulations we start with initial conditions where P from ATP is already confined to the microtubule. Then, we must impose a condition where the enzyme performs its barrier transfer activity. We impose that $\Gamma:0\rightarrow\Gamma_{\max}$ when $x_p<R_p$ and $r_{[p,\mathrm{ADP}]}>0.1$ nm. Then we observe how ATP hydrolysis is produced and after some time the head and the ADP bound together leave the microtubule. This time is crucial for the processivity, as the slower this process, the higher the processivity of the motor. As it is discussed in Ref. 3, the ATP binding on the attached head promotes ADP release on the other head, which causes the collapse of this head to the next tubulin site. This falling process has to be faster than the detachment of the trailing head to ensure that at least one head is always bound to the filament. Otherwise the motor would detach from the structure and the processivity would be lost. Then, the time for the attached head from ATP binding to head detachment in comparison with the time from ATP binding to the leading head collapse is essential to achieve a processive coordination between the heads. Next we will analyze an ATP hydrolysis process and then we will characterize this crucial time.

In Fig. 10 we can see the simulation performed for an ATP hydrolysis, previously schemed in Fig. 2. First of all, we

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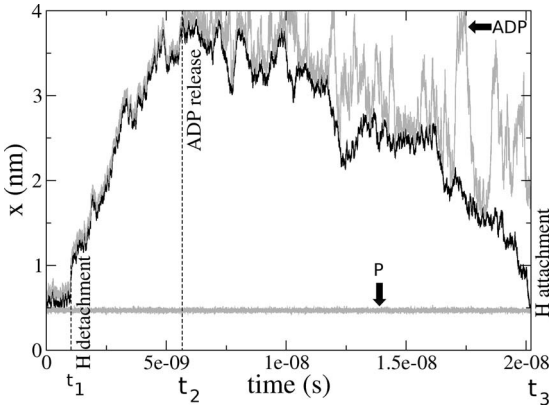

FIG. 10. Trajectories for a whole mechanochemical hydrolysis cycle in kinesin. The gray trajectory stabilized at $x \simeq 0.5$ nm corresponds to the position of the phosphate group P. The other gray trajectory corresponds to the ADP, while the black trajectory is the plot of the head trajectory. The initial conditions are located at $x_h=0.5$, $x_p=0.46$, and $x_{\mathrm{ADP}}=0.56$ nm, which correspond to an ATP which has already bound the attached head. In $t_1 \simeq 0$ s the Γ-state switches from 0 to $\Gamma_{\max}(=0.75)$. In $t \simeq 10^{-9}$ s the head detaches from the microtubule. The ADP and the head leave together the microtubule until the Γ-state of the head comes back to 0 due to an eventual ATP binding on the other head, which is not simulated here but given by hand (at $t_2 \simeq 5.7 \times 10^{-9}$ s, which should be much greater in a realistic situation). At $t_3 \simeq 2 \times 10^{-8}$ s the head has come back to the microtubule while the ADP remains far from the filament diluted in the bulk.

set the initial conditions with the ATP already bound to the tubulin site, since the time for the nucleotide to reach the P-confinement barrier in T is too long for a simulation with a time integration step of 10^{-16} s. Then the Γ-state switch occurs when $x_p < R_p$ and $r_{[p,\mathrm{ADP}]}$ is smaller than 0.1 nm. After this switch (which promotes the complementary Γ-state in the other head), the head detachment with the bound ADP occurs when a characteristic time (the necessary delay for the processivity) has passed. Then the head and the ADP leave the microtubule and travel due to diffusion and to the repulsion between the ADP$\cdot H$ complex and the negative microtubule surface. In the simulation, when the head passes through a position of $\sim$3.5 nm, we artificially switch the Γ-state. Then, the ADP is not strongly confined to the head anymore and this head starts an erratic trajectory to the microtubule again. Here we do not distinguish between different tubulin units because the directionality is given by the neck.[3] We can notice how the time left between the ATP binding and the head detachment is quite small, of the order of 1 ns. However, the time from the next Γ-switch until the arrival of the head to the microtubule is several times bigger. Under these circumstances, the motor would not be processive. What could make the initial time bigger in order to achieve processivity? If $\Gamma_{\max}$ is smaller than the value in Fig. 10 (0.75), the time for head detaching increases. If we set $\Gamma_{\max}=0.65$, the detaching time is about $\sim$2 ns, and $\Gamma_{\max}=0.55$ produces a time of $\sim$4 ns, but then the hydrolysis process is not so efficient and undesirable events where P, H, and ADP leave the microtubule are found with some probability. The reason for this is related to the value of E_Γ, which has the unrealistic value of 150 pN when in fact a more appropriate value should be much bigger. The problem is that very big values make the simulations very slow, as the potentials include huge forces. However, with higher values of E_Γ, the time delay would reach values that allow to explain processivity.

IV. CONCLUSIONS

The confinement effects between the interactions of P, ADP, kinesin head, and microtubule are responsible for the coordination of the mechanochemical hydrolysis cycle. Specifically, the ADP and P are able to bind in a stable way, the phosphate group is able to become confined to the microtubule, and the ADP is able to be confined to the head. These confinements, added to the electrostatic and steric interactions already discussed in our previous work,[3] allow to have a broad description of the whole mechanism. If in the previous work we discussed how the electrostatic changes in the head allow the hand-over-hand motion with a directionality given by the neck, now we have discussed how the nucleotide states are related to the affinity of the heads with the tubulin. We have given phenomenological expressions for the potential interactions between the four elements which are located in a 1D system for the sake of simplicity. Even though this is a strong simplification, the results that we obtained are in agreement with very recent results and allow to give some hints on the mechanisms that underlie the interaction between kinesin heavy chain and tubulin heterodimers.

Specifically, there are two major points that constitute the essence of our approach. On the one hand, the ability of the phosphate group to bind the microtubule plays a pivotal role in the understanding of the affinity of AMP-PNP bound heads for the microtubule and also gives a clue on how the phosphate release is related to head detachment. It was previously accepted that P_i release promoted head detachment,[4] but it was not clear from what did it detach. We understand that such a release means that P unbinds the ADP$\cdot H$ complex so this complex can leave the vicinity of the tubulin binding site. On the other hand, the hypothesis of the Γ commutator represents an assumption that is strongly phenomenological. The true nature of such interaction should be discerned experimentally. In order to check if the electric field of the ATP molecule influences the rate of ADP release in the detached head it could be interesting to utilize ATP analogs with extra negative charges or with no net charge and measure possible differences in the ADP release rate. Experiments with different levels of ionic strength and different tubulin isotypes and thus having different effective amounts of electric charge also could help understand the role of the electric fields in the head-head coordination. The effect of mechanical strain in such coordination should be tested by building different mutants with different values for the neck linker stiffness. The main goal would be to measure the degree of coordination of a loosely linked dimer.

ACKNOWLEDGMENTS

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