

1Diverse stepping motions of cytoplasmic dynein revealed by 2kinetic modeling

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13Short title

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15

16Abstract

17Cytoplasmic dynein is a two-headed molecular motor that moves to the minus end of
18microtubule (MT) using ATP hydrolysis free energy. By employing its two heads (motor
19domains), cytoplasmic dynein shows various bipedal stepping motions; the inchworm and hand-
20over-hand motions, as well as non-alternate steps of one head. However, the molecular basis to
21achieve such diverse stepping manners remains obscure. Here, we propose a kinetic model for
22bipedal motions of cytoplasmic dynein and performed Gillespie Monte Carlo simulations that
23reproduces most experimental data obtained to date. The model represents status of each motor
24domain as five states according to conformations, nucleotide- and MT-binding conditions of the
25domain. Also, the relative positions of the two domains were approximated by three discrete
26states. Accompanied by ATP hydrolysis cycles, the model dynein stochastically and
27processively moved forward in multiple steps via diverse pathways, including inchworm and
28hand-over-hand motions, same as experimental data. The model reproduced key experimental
29motility-related parameters including velocity and run-length as functions of ATP concentration
30and external force. Our model reveals that, in a typical inchworm motion, the leading domain
31moves via the ATP-dependent power-stroke of the linker coupled with a small change in the
32stalk angle, whereas the lagging domain moves via diffusion dragged by the leading domain.
33Moreover, the hand-over-hand motion in the model dynein clearly differs from that of kinesin
34by the usage of the power-stroke.

35Author Summary

36Cytoplasmic dynein is a two-headed molecular motor, which moves linearly and transports
37intra-cellular organelles along microtubules driven by ATP hydrolysis free energy. In contrast to
38other better-known molecular motors, such as kinesin, dynein is known to take various stepping
39motions including motions akin to human walking and inchworm-like motions. However,
40molecular mechanisms underpinning the diverse stepping motions are unclear. Here, based on
41recent high-resolution structure information and single-molecule motility assay data, we
42designed a kinetic model that explicitly include two heads, each of which makes ATP
43hydrolysis cycles and moves along the microtubules. Using the model, we performed Monte
44Carlo simulations. The simulation reproduced most of currently available experimental results.
45More importantly, the simulation suggested molecular mechanisms of various stepping motions.
46While stepping motions apparently resemble to those proposed before, once looking into details,
47we found the resulting mechanisms distinct from previously proposed ones in the usage of ATP
48and protein conformation changes coupled with stepping motions.

49

50 Introduction

51 Cytoplasmic dynein (hereafter, denoted as dynein for simplicity) is a molecular motor that show
52 bipedal motions on the microtubules (MT) to its minus end, driven by ATP hydrolysis free
53 energy[1–3]. This motility enables dynein to play essential roles in various cellular functions
54 including intracellular transport, positioning of organelles and cell division. As such, dynein is
55 often compared with another MT-based molecular motor, kinesin, most of which walks to the
56 plus end of MT[4]. Single-molecular measurements clarified that the kinesin-1 walks rather
57 regularly via the so-called hand-over-hand manner; two motor domains alternately move from
58 the lagging position to the leading position, akin to human walking. This motion of kinesin
59 leads to precise 8 nm steps per one ATP hydrolysis reaction[5]. The hand-over-hand mechanism
60 is also in harmony with the experiment that a mutant kinesin that impairs one motor domain
61 severely slows down kinesin motility [6,7]. In contrast, dynein moves more stochastically with
62 various step size, which ranges 4-32 nm with its representative size of 8 nm [8–11]. In addition
63 to the hand-over-hand motions, dynein can take the so-called inchworm-like motions; walking
64 via alternating steps with one motor domain being always ahead of the other domain, as well as
65 non-alternating steps[10–13].

66 Previous structural studies delineate the ATP-dependent conformational changes in each
67 dynein motor domain. The dynein motor domain consists of the ATPase associated with diverse
68 cellular activities (AAA+) ring[14,15], the microtubule binding domain (MTBD), the stalk, the
69 linker, and the tail domain (see cartoons in Fig 1A). The AAA+ ring (a large donut shape in Fig
70 1A) made of six subdomains, AAA1-AAA6, possesses the ATPase activity and energizes the
71 dynein movement[16]. The MTBD (small circles near the MT in Fig 1A) is a small domain
72 responsible for the binding to MT. The stalk is a long coiled-coil that connects the AAA+ ring
73 and the MTBD[16,17]. The linker (an arrowhead-like object drawn on the AAA+ ring) is
74 associated with the AAA+ ring and connects to the tail domain where two motor domains
75 dimerize (the tail domain not drawn in Fig 1A)[18,19]. Biochemical and structural experiments
76 have revealed that ATP hydrolysis reactions in AAA1 play the primary role in the movement,
77 affecting conformations of the entire motor domain[15,20]. In particular, depending on the
78 nucleotide state in AAA1, both the linker and the MTBD make marked structural changes
79 which are of central importance in the motility[17]. When AAA1 is in the ATP-bound state, the
80 linker is largely bent on the AAA+ ring and the tip of the linker is located near AAA2/AAA3
81 subdomains (the bottom-left cartoon in Fig 1B), whereas, in the ADP bound and apo state of
82 AAA1, the linker tends to be more extended on the AAA+ ring reaching to the AAA4/AAA5
83 subdomains (the top-right cartoon in Fig 1B)[21–24]. The linker is expected to swing

84simultaneously or immediately after Pi release and swings back upon ATP binding, which are
85termed the power-stroke and recovery-stroke, respectively. The power-stroke of the linker is
86considered to be responsible for dynein force generation [17,25,26]. The MTBD tends to take a
87low affinity state to MT in the ATP bound state, while it takes a high affinity conformation to
88MT in the ADP-bound and apo states [20]. This ATP-dependent change in the affinity of
89MTBD must be critical, because dynein needs to bind tightly with MT when it exerts force
90against MT but needs to dissociate from MT during recovery-stroke to avoid backward
91movement[27,28]. In addition to these specific parts of the motor domain, allosteric
92conformational changes in the entire motor domain may play an important role for the dynein
93movement[29,30].

94

95**Fig 1. The kinetic model of the two-headed dynein.** (A) Architecture of the dynein motor
96domain structure. (B) The five state model of one dynein motor domain and transitions among
97states. (C) The motor domain position. The position is defined by the tip of the linker where
98cargos and/or beads are attached. In the model, it takes discrete positions with 8 nm spacing. (D)
99The dimeric dynein state. The first (designated red) motor domain is either 8 nm ahead of, at the
100same position as, or 8 nm behind the second (blue) motor domain, which are represented,
101respectively, as +, 0, or -. As a whole, there exist $5 \times 5 \times 3 = 75$ states for the bipedal dynein.
102We label each state, e.g., 2TDM+ where the first integer is the state number from 1 to 75,
103followed by the states of the red and then blue motor domains. The rightmost symbol represents
104the positioning of the red domain relative to the blue. (E) Effects of external force to the
105backward direction on transition rates. (F) Effects of internal force/tension on transition rates.

106

107 However, these ATP-dependent conformational changes in each motor domain alone do not
108explain how diverse walking manners are realized. In order to clarify the mechanism of various
109walking manners, we need to characterize coordination of the two motor domain movements
110coupled with ATPase cycle. Observing the coordinated motion directly by single-molecule
111experiments is, however, currently difficult due to the time- and the spatial-resolution.

112 Therefore, several theoretical models have been proposed to understand the walking
113mechanisms of dynein. Most theoretical models proposed before the X-ray structure reports are
114unavoidably simple and consider only one motor domain [31,32]. A more elaborate kinetic
115model that explicitly deals with coordination of the two motor domains clarified a class of
116necessary coordination and the force-dependent motility change well [33]. The model is,
117however, limited to the hand-over-hand coordination and is not compatible with recent
118experimental data. More recently, a mechano-chemical model that connects the two ATP-

dependent motor domains via elastic bonds elucidated correlation between the motion and the tension [34]. Yet, in the model, chemical cycles in the two domains are treated as independent. However, in order to clarify the mechanism of versatile walking mechanisms, it is crucial to model how ATP hydrolysis reactions and conformational change proceed, correlated with the relative configuration of the two motor domains.

In this study, we propose a new kinetic model of dynein that explicitly contains chemical and conformational states of each motor domain, directly coupled with the relative positions of the two domains along MT. The kinetic model can explain molecular basis of versatile walking manners. We represent chemical and conformational states of each dynein motor domain as 5 discrete states. For the relative positions of the two domains, for simplicity, we assume it taking 3 discrete states: either one is 8 nm ahead of, at the same position as, or 8 nm behind the other motor domain along MT. Together our kinetic model consists of $5 \times 5 \times 3 = 75$ states and considers transitions among these states, which is solved by Monte Carlo (MC) simulations with the Gillespie algorithm[35]. We reproduced experimentally observed behaviors with various dynein walking manner and uncovered detailed and comprehensive pathways.

134

135 **Model and Methods**

136 **Five state model for a dynein motor domain.**

Our model assumes that each dynein motor domain takes one of five possible states depending on the combination of the nucleotide bound in AAA1, the binding to MT, conformation of the linker, and that of MTBD (Fig 1B). The five states are the followings:

- 140(1) The DM state: ADP is bound in AAA1. The linker is in the post-power-stroke (extended) state. The high-affinity MTBD binds to MT.
- 142(2) The T state: ATP is bound in AAA1. The linker is in the post-power-stroke state. The low-affinity MTBD is unbound from MT.
- 144(3) The T* state: ATP is bound in AAA1. The linker is in the pre-power-stroke (bent) state. The low-affinity MTBD is unbound from MT. The stalk leans towards the MT long axis.
- 146(4) The D*M state: ADP is bound in AAA1. The linker is in the pre-power-stroke state. The high-affinity MTBD binds to MT. The stalk leans towards the MT long axis.
- 148(5) The D state: ADP is bound in AAA1. The linker is in the post-power-stroke state. The high-affinity MTBD is, however, unbound from MT.

Of the five states, the states (1) and (3) correspond to X-ray crystal structures solved to date (PDB ID: 3VKH and 4RH7, respectively)[22,36] (note that those are for different families) and thus are assumed to be relatively stable, whereas the other three states are considered as

transient states with higher free energies. We note that, when the linker takes the pre-power-stroke state (the D*M and T* states), we assume that the stalk leans to the MT long axis with a smaller angle between the stalk and the MT compared with the other states. This is based on experimental data that suggests the motor domain to stand up with the MTBD position fixed upon power stroke motion[37]. This suggestion was further supported by some electron-microscopic observations for cytoplasmic dynein [38,39] as well as for axonemal dynein [17].

Next, we setup transitions among the five states of motor domain.

(1) DM → T: ADP release is followed by ATP binding and a dissociation of the motor domain from MT

(2) T → T*: The recovery-stroke of the linker

(3) T* → D*M: ATP hydrolysis is followed by the Pi release and the binding of the motor domain to MT

(4) D*M → DM: The power-stroke of the linker

The above four transitions form the main (and thus productive) ATP hydrolysis cycle.

Additionally, we include the following off-pathways.

(5) D*M → D & DM → D: The dissociation of the motor domain from MT

(6) D → T: ADP release is followed by ATP binding, without the binding to MT

(7) T* → D: ATP hydrolysis is followed by Pi release, without the binding to MT.

Our model assumes that the ATP hydrolysis cycle of one ATP molecule corresponds to one power-stroke and recovery stroke, without considering ATP hydrolysis in other AAAs than AAA1 subdomain.

Since the dynein motor domain never synthesize ATP from ADP and Pi in any condition, we do not consider the reverse transitions of (3) or (7), D*M → T* or D → T*. For all the other transitions, we take into considerations of the reverse transitions. We note that all the cyclic transition paths that do not include the ATP hydrolysis, i.e. T* → D*M and T* → D, should give no net free energy changes. This leads us to the following equalities to be satisfied.

$$k_{D^*M \leftarrow D} \cdot k_{DM \leftarrow D^*M} \cdot k_{D \leftarrow DM} = k_{D \leftarrow D^*M} \cdot k_{D^*M \leftarrow DM} \cdot k_{DM \leftarrow D} \quad (1)$$

$$k_{D^*M \leftarrow D} \cdot k_{DM \leftarrow D^*M} \cdot k_{T \leftarrow DM} \cdot k_{D \leftarrow T} = k_{D \leftarrow D^*M} \cdot k_{D^*M \leftarrow DM} \cdot k_{DM \leftarrow T} \cdot k_{T \leftarrow D} \quad (2)$$

$$k_{DM \leftarrow D} \cdot k_{T \leftarrow DM} \cdot k_{D \leftarrow T} = k_{D \leftarrow DM} \cdot k_{DM \leftarrow T} \cdot k_{T \leftarrow D} \quad (3)$$

Movement of dynein motor domain along MT

We next define the position of the dynein motor domain. Since cargos *in vivo* and beads in single-molecule experiments are connected to the tip of the linker, we define the position of

186motor domain by the position of the tip of the linker. In addition, we approximate that the tip of
187the linker resides in discrete positions spaced with 8 nm (Fig 1C).

188 Next, we discuss the movement of the motor domain. We assume that the position of the
189linker tip changes via the power-stroke/recovery-stroke, and the diffusive motion. First, in the
190power-stroke transition $D^*M \rightarrow DM$ (see Fig 1B), the tip of the linker position moves 8 nm to
191the forward direction (the minus end of MT), whereas in the reverse reaction of the power-
192stroke (note that this is not the recovery stroke), the tip moves 8 nm to the backward direction
193(the plus end of MT) although the reverse reaction rarely happens.

194 Both the yeast and human dynein molecules can move forward against 5~10 pN backward
195loading [9,40]. If the load force disturbs the linker motion completely, the dynein molecules
196would not step forward. Therefore, as a common feature of dynein, the power-stroke motion of
197the linker should be able to undergo against more than 5~10 pN of the load. This led us to
198model the following energy difference between pre- and post- power stroke states;

$$199 \frac{k_{DM \leftarrow D^*M}}{k_{D^*M \leftarrow DM}} = \exp\left(\frac{10 \text{ pN} \times 8 \text{ nm}}{k_B T}\right)$$

200where T is the temperature, the Boltzmann constant k_B takes $k_B = 0.0138 \text{ pN} \cdot \text{nm} / K$. We note
201that the tip of the linker does not move during the recovery-stroke from the T to T* states
202because the MTBD is unbound from MT.

203 Next, we consider the diffusive motion. In our five states model, only in the D, T and T*
204states among five states of our model, the motor domain can move diffusively along MT. We
205assume that the motor domain takes an 8-nm step to forward or backward direction during the
206diffusive movement. We define the rates for the forward and backward diffusive transitions as
207 λ_{for} and λ_{back} , respectively. The values of λ_{for} and λ_{back} are set as the same for all the three
208states; the D state, the T state, and the T* state. In addition, by symmetry, λ_{for} and λ_{back} must be
209the same values when the external force is not applied.

210

211 **The dimeric dynein motor domains**

212We assume that dynein moves as a homo-dimer of the motor domain throughout this study. To
213distinguish two identical motor domains, throughout the paper, we call the first one as "red
214domain" and the second domain as "blue domain", merely for convenience, corresponding to the
215colors used in figures (Fig 1D for example). For the minimal complexity of the model, we set
216that the difference in the positions of the two motor domains along MT being either +8 nm, 0
217nm, or -8 nm (Fig 1D for the case of +8 nm). Thus, the relative positions of the two dynein
218motors can be regarded as the three-state model. When the red motor domain is ahead of, at the

219 same position as, and behind the blue motor domain, we write their configurations as +, 0, and -,
220 respectively (The minus end of MT is regarded as the forward direction). Assumed here is that
221 the two motor domains use different protofilaments so that they can have 0 nm distance along
222 axial direction of MT. We do not include the side-steps from one to another proto-filaments. We
223 also note that the generalization of this restraint of the inter-motor-domain distance to the
224 multiples of 8 nm is straightforward.

225 Combining 5 states for each motor domain and 3 states for the relative positions between the
226 two motor domains, the current model for the dimeric dynein has $5 \times 5 \times 3 = 75$ states in total.
227 To indicate each of 75 states clearly, we introduce a combinatorial notation, which we explain
228 with an example, 2TDM+. In this notation, the first integer (2 in the example) represents the
229 numbering of the state from 1 to 75 (S1 Table). The following characters represent states of the
230 first (red one in Fig 1D) and the second (blue one in Fig 1D) motor domains; in this example the
231 red motor domain is in the T state, while the blue one is in the DM state. The last symbol, either
232 +, 0, or -, represents the relative position of the two motor domains along MT.

233

234 Effect of external forces

235 Our model incorporates the effect of external force. We suppose the external force is applied to
236 the tip of the linker through the attached bead or cargo in the direction parallel to MT. In this
237 work, we limit ourselves to the case where the external force is applied to the plus end
238 (backward direction to the functional movements) of MT. When one motor domains is ahead of
239 another domain, i.e. the state with + or -, it is natural to assume that the external force is applied
240 only to the motor domain that locates in the opposite side to the direction of the external force.
241 Thus, the external force F is applied to the leading motor domain located closer to the minus
242 end. When the two motor domains take the same position along the MT, we assume each motor
243 domain is subjected to a half of the external force $F/2$.

244 Since the power-stroke changes the bead position, transition rates for the power-stroke and its
245 reverse process must be affected by the external force. We can easily incorporate this effect into
246 the energy difference between pre- and post- power-stroke states as,

$$247 \frac{k_{DM \leftarrow D^i M}}{k_{D^i M \leftarrow DM}} = \exp\left(\frac{(10 - F) pN \times 8 nm}{k_B T}\right) \quad (4)$$

248 Of note, the external force F takes the positive value when dynein is pulled to the plus end
249 direction of MT. Based on this equation, we rescaled the rate of the reverse of the power-stroke
250 process, making it more probable to occur as the force is applied, whereas the rate of the power-
251 stroke is unchanged.

252 Similarly, the diffusive motions are affected by the external force. It is natural to assume the
253 ratios between λ_{for} and λ_{back} depends on the external force as

$$254 \frac{\lambda_{for}}{\lambda_{back}} = \exp\left(\frac{-F \text{ pN} \times 8 \text{ nm}}{k_B T}\right) \exp(-1.9 F \text{ pN}) \quad (5)$$

255 We have multiple choices of λ_{for} and λ_{back} that satisfy this equation. Here, we put the force de-
256 pendence in λ_{back} making the backward diffusion more probable to occur as the force is ap-
257 plied, while the rate of the forward step is unchanged. Besides, previous experiments uncovered
258 that the dissociation rates of the motor domain from the MT increases with the external force
259 [41,42]. Since this could be an essential feature of the dynein motor domain, we include this ef-
260 fect into our model. Specifically, all the transitions from MT-bound states to unbound states,
261 such as $D^*M \rightarrow D$ and $DM \rightarrow D$, that occur in the leading motor domain are accelerated by a
262 force-dependent factor. Note that this corresponds to the increase in the free energy of the lead-
263 ing domain MT-bound state by the logarithm of the force-dependent factor. We introduce a
264 multiplying factor $f_{ext}(F)$ into the dissociation rates of the motor domain under the force F . An
265 example is illustrated in Fig 1E. We set $f_{ext}(F) = \exp(B \cdot F)$ because the associated energy
266 changes should be proportional to the external force. Here, B is a fitting parameter that we esti-
267 mate below. When the motor domain that is subjected to a half of the external force dissociates
268 from MT, the factor becomes $f_{ext}(F/2) = \sqrt{f_{ext}(F)}$ (see an example in Fig 1E from the third to
269 the fourth states).

270

271 Internal force between two motor domains

272 Since the two motor domains are connected, each motor domain should receive internal force
273 from the other one when the both motor domains are bound on MT. We treat the internal force
274 being independent of the external force. Previous experimental study shows the asymmetric
275 dissociation rates of MTBD from MT for the case of internal force [41,42]. Therefore, the
276 lagging motor domain should dissociate with larger rates than that of the leading (minus-end
277 side) motor domain due to the opposite directions of internal force for the leading and lagging
278 motor domains when both motor domains are bound to MT with 8-nm gap,. We incorporate this
279 effect by introducing a multiplying factor f_{int} into the dissociation rate of the lagging motor
280 domain when the leading domain is also bound on the MT (Fig 1F).

281 To satisfy the condition that the free energy change along any cyclic path must be zero, we
282 put the same factor f_{int} , either in the numerator or in the denominator, of many surrounding

transition rates, as in S1 Fig. We begin with the acceleration of the transitions by f_{int} ; $4D^*MDM^+ \rightarrow 24D^*MD^+$ and $1DMDM^+ \rightarrow 21DMD^+$ (see Fig 1F), in which the lagging motor domains are peeled out due to the inter-motor domain force. These changes are accommodated by increasing the free energies of $4D^*MDM^+$ and $1DMDM^+$ by $\ln f_{\text{int}}$, which can be viewed as the internal tension. These free energy changes in the two states result in including the same multiplying factor, either in the numerator or in the denominator of all the rate constants connected with these two states (Fig 1F for an example and S1 Fig for the complete picture). For example, in S1 Fig, $4D^*MDM^+$ can transit to $5DDM^+$ and $9D^*MT^+$ so that we introduce f_{int} into the incoming rates from these two states.

292

293 Setting up the rate constants

294 Now, we discuss the values of rate constants. As of now, due to the lack of experimental measurements, we cannot decide many parameters without uncertainty. Yet, we would like to choose a set of parameters that satisfy the physics law, equations (1)-(4), and that are consistent with all the available data as much as possible.

298 First, from single-molecule experiments for the monomeric dynein, we can set

$$299 k_{T \leftarrow T} = 200 \text{ s}^{-1}, k_{D^*M \leftarrow T} = 200 \text{ s}^{-1}, k_{D \leftarrow T} = 5 \text{ s}^{-1} [43].$$

300 Second, the transition $DM \rightarrow T$ contains the ADP release ($k_{ADP \leftarrow \text{int}}: 10 \text{ s}^{-1}$), ATP binding ($k_{ATP + \text{int}}: 2 \mu M \cdot \text{s}^{-1}$), and the dissociation from MT ($k_{\text{off}}: 500 \text{ s}^{-1}$), as a roughly sequential process [43]. So we can express $k_{T \leftarrow DM}$ as

$$303 k_{T \leftarrow DM} = \frac{20 \cdot [ATP]}{2 \cdot [ATP] + 10}$$

304 In the derivation process, we assumed that the dissociation from MT is much faster than the other processes. Thus, $k_{T \leftarrow D}$ becomes the same as $k_{T \leftarrow DM}$.

306 Third, since direct experimental data are not available at the moment for other values, we infer physically feasible values guided by the detailed balance conditions and other restraints. We assume the power-stroke motion is fast enough and set it as

309 $k_{DM \leftarrow D^*M} = 1000 \text{ s}^{-1}$. Of note, as far as this is large enough, the result does not depend on this precise value at all. Using the physical constraint, eq.(4), we also can obtain

$$311 k_{D^*M \leftarrow DM} = 1000 \exp\left(\frac{-(10 - F) pN \times 8 nm}{k_B T}\right)$$

Next, we suppose that the high affinity MTBD does not dissociate from MT without the applied external or internal force. We thus choose $k_{D \leftarrow DM}$ as a sufficiently small value; specifically, it must be much smaller than $k_{T \leftarrow DM} \cdot 10 \text{ s}^{-1}$ at its saturated value. Since both $k_{D \leftarrow DM}$ and $k_{D \leftarrow D^*M}$ represent dissociation of the high affinity MTBD from MT, we set $k_{D \leftarrow DM} = k_{D \leftarrow D^*M}$. The D state must be inherently unstable. Thus, the lifetime of the D state is short and it changes quickly to the DM state. It is natural to assume $k_{DM \leftarrow D} > k_{T \leftarrow DM} \cdot 10 \text{ s}^{-1}$. From the detailed balance condition, we get

$$k_{D^*M \leftarrow D} = k_{DM \leftarrow D} \exp\left(\frac{-(10-F) \text{ pN} \times 8 \text{ nm}}{k_B T}\right)$$

Others rate constant values are determined to satisfy the detailed balance. Finally, $k_{T \leftarrow T^*}$ should be smaller than the reverse process, $k_{T^* \leftarrow T}$.

We list all the kinetic parameters used in the current simulations in Table 1.

Table 1 Rate constant values for motor domain

Rate constant	values (s^{-1})
$k_{T^* \leftarrow T}$	200
$k_{D^*M \leftarrow T^*}$	200
$k_{DM \leftarrow D^*M}$	1000
$k_{T \leftarrow DM}$	$\frac{20 \cdot [ATP]}{2 \cdot [ATP] + 10}$
$k_{T \leftarrow T^*}$	10
$k_{DM \leftarrow T}$	1/10
$k_{D^*M \leftarrow DM}$	$1000 \exp\left(\frac{-(10-F) \text{ pN} \times 8 \text{ nm}}{k_B T}\right)$
$k_{D \leftarrow D^*M}$	1/100
$k_{D^*M \leftarrow D}$	$100 \exp\left(\frac{-(10-F) \text{ pN} \times 8 \text{ nm}}{k_B T}\right)$
$k_{D \leftarrow DM}$	1/100
$k_{DM \leftarrow D}$	100
$k_{D \leftarrow T}$	1/100000
$k_{T \leftarrow D}$	$\frac{20 \cdot [ATP]}{2 \cdot [ATP] + 10}$
$k_{D \leftarrow T^*}$	5
λ	200

The unit of [ATP] is μM .

326

327 Next, we determine the external force dependent factors. The detachment force of dynein,
328i.e., a critical force beyond which dynein detaches from MT promptly, to the plus end of MT
329with the high affinity MTBD is estimated to be approximately 2 pN (Shima et al, manuscript in
330preparation). This suggests that, with the external force of 2 pN, the DM state should change to
331the D state, rather than the other states, i.e., the T state. Thus, we require

332 $k_{T \leftarrow DM} \ll f_{ext}(F) \cdot k_{D \leftarrow DM} (k_{D \leftarrow D^+M})$. We note that $k_{T \leftarrow DM} = 10 \text{ s}^{-1}$ at the saturated ATP con-
333centration. Since we set the dissociation rates with no external force to be

334 $k_{D \leftarrow D^+M} = k_{D \leftarrow DM} = \frac{1}{100} \text{ s}^{-1}$, previously, we decided to choose $f_{ext}(2 \text{ pN}) = 10000$. To this

335end, we obtain $f_{ext}(F) = 100^{(F \text{ pN})} \exp(4.61 F \text{ pN})$. Note that the estimate of the detachment
336force contains some uncertainty; if the detachment force were equal to 3 pN, we would have

337 $f_{ext}(F) \exp(3.07 F \text{ pN})$

338 While there is no experimental estimate for the value of f_{int} , merely for the consistency with
339the effect of the external force in the previous section, we set $f_{int=10000}$; we suppose the dissoci-
340ate rate of the relevant motor domain from MT may be equal. We also tested how this value
341would alter the results, finding that most of the qualitative results are not changed, except the
342probabilities to choose individual paths (see Discussions).

343

344 Monte Carlo simulations

345As the basic dynamic equations for the kinetic model of $5 \times 5 \times 3 = 75$ states, we take the master
346equation;

347 $\frac{d p_i(t)}{dt} = \sum_{j=1}^{75} T_{i \leftarrow j} p_j(t)$ where $p_j(t)$ is the time-dependent probability to be in the j-th state and

348the transition matrix $T_{i \leftarrow j}$ represents corresponding transition rates for the non-diagonal
349elements and cumulative outgoing rates for the diagonal elements, respectively. Of the 75×75
350matrix elements, we have already described all the non-zero elements, while the other elements
351are zero. In this work, we solved this master equation via Gillespie Monte Carlo (MC)
352simulations, a random number-based sampling of the solution. When the system resides in j-th
353state at a time t, the Gillespie algorithm first chooses the state to go; among those connected
354from the current state j, a state i is chosen by the probability proportional to the corresponding
355transition rate $T_{i \leftarrow j}$. Next, using the summation of all the rates connected from the state j,

356 $k_j = \sum_i T_{i \leftarrow j}$, the transition time Δt is drawn randomly from the waiting time distribution in
 357 Poisson process, $P(\Delta t) = k_j \exp(-k_j \Delta t)$. In all the MC trajectories, we begin with the initial
 358 state, 1DMDM+, the red motor domain in the DM state is ahead of the blue motor domain,
 359 which is also in the DM state. Each MC simulation is terminated at the moment when both
 360 motor domains are disassociated from MT (i.e., T, T*, or D state) or when the time exceeds 10s.
 361 For each setup, we repeated MC simulation 10000 times with different random number
 362 sequences.

363 We also note that the current master equation can be solved by the standard linear algebra,
 364 as well. We tried to solve the linear algebraic equation by an algebraic manipulation software.
 365 However, we did not succeed to obtain the closed formula for this large matrix. Still, one can
 366 solve the linear equation numerically, obtaining the exact numerical values for velocity and the
 367 run length. However, the MC simulation is more straightforward to analyze pathways, and thus
 368 we took the MC approach in this study.

369

370 Analysis of the run length from trajectory

371 When we determine the run-length from a trajectory, to avoid possible artefacts in the initial
 372 configuration, we used a scheme used in experiments[38]. First, we plotted the cumulative
 373 probability distribution $c(x)$ (red crosses in Fig 2B) as a function of the run length x , and
 374 confirmed that $1 - c(x)$ exhibits exponential decay for $x > 1$ step. This is probably due to the
 375 Poisson process of the dynein detachment. Given the exponential behavior, we fitted $c(x)$ with
 376 the form,

$$377 c(x) = 1 - \exp\left(-\frac{x - x_0}{x_{mean}}\right)$$

378 to obtain x_0 and the mean run length x_{mean} by the nonlinear least square estimation with the
 379 Marquardt-Levenberg algorithm. Here, x_0 is used to accommodate irregular behavior of the
 380 very initial stage.

381

382 **Fig 2. Bipedal motions of dimeric dynein via Monte Carlo simulations.** (A) Five
 383 representative trajectories at [ATP] = 1mM with no external force. The red and blue curves,
 384 respectively, represent positions of red and blue motor domains along MT in one trajectory.
 385 Grey curves represent movements of two motor domains in four other trajectories. The inset is a
 386 close up between 0.6s and 0.8s. (B) The cumulative probability distribution (red crosses)
 387 calculated from the final arrival distance for 10000 MC trajectories under the same condition as

388(A) and its fitted curve (green). (C) Median velocity and mean run length as a function of [ATP]
 389with no external force. Error bars from each point of x_{mean} are asymptotic standard errors. (D)
 390Mean velocity as a function of the external force for a few different [ATP]. (E) Median number
 391of steps per consumed ATP (ATP efficiency) for a few different [ATP] and external forces. The
 392error bar represents quartile range. (F) Histograms of the hand-over-hand and inchworm
 393motions.

394

395

396 Results

397 Kinetic model reproduces many data on the wild-type dynein motility

398 First, we examine the basic motility of our kinetic model comparing the simulation results with
 399 experimental data. We performed MC simulations of wild type dimeric dynein movement with
 400 [ATP] = 1 mM and no external force. We repeated simulations 10,000 times, from which five
 401 representative trajectories are shown in Fig 2A (see S2 Fig for the entire trajectories). For this
 402 trajectory, we see that dynein stochastically proceeded to the minus-end direction of MT for ~20
 403 steps before detachment from MT at ~2.2s. Interestingly, we find dynein sometime moved quite
 404 rapidly, but also exhibited occasional pauses. During the pauses, we find some rapid stamping
 405 of one motor domain (while one domain pauses in a position, the other domain moves back and
 406 forth many times, as in the inset of Fig 2A). The other four trajectories (drawn in grey curves in
 407 the figure) proceeded to the same direction and roughly with the same velocity.

408 From the 10,000 trajectories, we estimated the mean run length; for example, for the case of
 409 [ATP] = 1 mM, it was estimated as 3.6 ± 0.15 step (Fig 2B). We plot the mean run length as a
 410 function of the ATP concentration [ATP] (Fig 2C). The mean run length shows a peak at around
 411 [ATP] = 1 μ M and levels off at higher [ATP]. The ATP-bound dynein, the T or T* states, has
 412 weak affinity to MT and thus the saturated [ATP] diminish the run length. The characteristic
 413 event that the run-length becomes longer with lower [ATP] was also reported in 22S dynein of
 414 Tetrahymena cilia and cytoplasmic dynein of mammalian [44,45].

415 We then plot the velocity as a function of the ATP concentration [ATP] (Fig 2C). For the
 416 velocity, we defined it as the ratio of the distance of movement to the time duration between
 417 long-time pauses, from which we obtained the median v_{med} of the velocity distribution as the
 418 representative value. We note there can be several other ways to define the velocity, which we
 419 discuss in the supporting information (S3 Fig). As [ATP] increases, the velocity increases at the
 420 low [ATP] and then saturates at higher [ATP], which is qualitatively consistent with
 421 experiments. In the saturated [ATP], each motor domain is bound by ATP for most of time and

the ATP binding does not determine the velocity, as usual. The maximum velocity in the model dynein was about 6 step/s $\sim$ 50 nm/s.

Experimentally, the velocity of dynein movement has been measured and known to depend on the species/constructs. The velocities for the full length and the GST dimer of *Dictyostelium* dynein are $\sim$ 200 nm/s [46], and $\sim$ 500 nm/s [47], respectively. For mammalian dynein, the DDB complex proceeds with 500 $\sim$ 800 nm/s and the GST dimer velocity is $\sim$ 500 nm/s [48]. For yeast, the full length dynein moves at the velocity $\sim$ 80 nm/s [8] and the GST dimer proceeds with $\sim$ 100 nm/s [49]. Our model dynein moved with the velocity similar to that of yeast dynein. Reasons that the model shows lower velocity than *Dictyostelium* dynein may be attributed to the choice of the low rate constant for $k_{T \rightarrow DM}$ which actually could dramatically change depending on the dimeric state [43].

Next, we calculated dynein motions under the external force to the backward direction, i.e., towards the plus-end of MT. The estimated velocity v_{med} is plotted against the strength of the external force for a few different [ATP] in Fig 2D. With non-zero [ATP], the velocity is positive at low external force and becomes negative at sufficiently large force, as expected. Notably, the mean velocity crosses zero at the same external force ($\sim$ 2 pN), regardless of [ATP]. This is in harmony with a recent experimental result in dynein motility assays (Shima et al, manuscript in preparation). Another non-trivial behavior is a slight increase in the velocity with a weak external force of 0 - 1 pN, which we will discuss later.

We also estimated the efficiency of our model dynein. Specifically, we plotted in Fig 2E the median forward step numbers per one ATP hydrolysis, termed ATP efficiency for brevity, for several combinations of [ATP] and the external force. Focusing on the ATP efficiency in the absence of external force, although the run length and the velocity differ by far between [ATP] = $1 \mu M$ and [ATP] = $10^3 \mu M$, the ATP efficiency changes less than 5%. Namely, the average move per one ATP cycle does not depend on the ATP concentration much, whereas the waiting time for ATP binding depends on [ATP] and thus affects the velocity at low-to-medium range of [ATP]. As [ATP] increases, the ATP efficiency decreases slightly from 0.71 at $1 \mu M$, to 0.68 at $10^3 \mu M$. This is because the forward move by the power-stroke is partly canceled by the influence of diffusion in the weakly coupled dissociation state. When we applied the external force to the backward direction, we found the increase in the ATP efficiency for the range [ATP] = $1 - 10^3 \mu M$ and up to the force of 1 pN (the efficiency reaches to $\sim$ 1). Above 1.5 pN, the efficiency decreased. This is related to the increase in the velocity with a weak external force mentioned above, and we will come back to this feature in the pathway analysis later.

Notably, with large external forces (1.5 pN and above for $[ATP] = 1 \mu M$, and 2.0 pN for the other $[ATP]$), we found a much larger variance in the ATP efficiency than the cases of weaker force. With large opposing force, dynein occasionally goes backwards largely and then detaches from MT, which makes sampling of broad data difficult.

460

461 **Mutants that impair one motor domain activity**

It is an interesting feature of dimeric dynein that it can proceed to the same direction even when one of motor domains lacks the ATP binding or hydrolyzing ability [11,50]. Here, to test our model, we performed MC simulations for the two cases that mimic the two types of impaired heterodimeric mutants. Fig 3A shows representative trajectories for the case where one motor domain does not bind ATP. Clearly, with the ATP-binding deficient mutant in one motor domain, it still moved to the forward direction with qualitatively similar processivity. Not surprisingly, during the processive movement, the intact motor domain was ahead of the mutant motor domain in most steps. We also see this mutant showed slightly reduced velocity and slightly increased run length, compared to the wildtype (grey in the figure) (Fig 3C and 3D). This is because the mutation leads to increase the population in the high-affinity state to MT, which slowed the movement and increased the processivity. Both of these effects have been shown in previous experiments, suggesting our model calculation qualitatively agreed with the experiments [50].

475

Fig 3. Motility of two mutants of dynein. (A) Representative trajectories at $[ATP] = 1 mM$ with no external force for a mutant of which right (blue) motor domain does not bind ATP. The positions of the deficient motor domain are drawn in black. Grey trajectories are for wild-type. The inset is a close up between 3s and 3.5s. (B) Representative trajectories at $[ATP] = 1 mM$ with no external force for a mutant of which right (blue) motor domain does not hydrolyze ATP. The positions of right motor domain are drawn in black. Grey trajectories are for wild-type. The inset is a close-up view from 0s to 0.14s. (C) Histogram of the run length. Red, green, and blue curves represent the wild-type, the case where one motor domain does not bind ATP, and the case where one motor domain does not hydrolyze ATP, respectively. (D) Mean velocities and their standard deviations for the three cases as in (C).

486

487

Fig 3B plots trajectories for the case where one of motor domain does not hydrolyze ATP. The ATP-hydrolysis deficient mutant moved forward, but markedly reduced the processivity

and run length because the mutated motor domain stay in T or T* states with low affinity to MT. We also see this mutant showed slightly reduced velocity, compared to the wild-type case (grey in the figure) (Fig 3C and 3D). Indeed, previous experiments showed decreases in the run-length and the velocity for this mutant [50]. Thus, our model calculation qualitatively agrees with the experiment.

We note, however, that experiments showed the ATP-hydrolysis deficient mutant moves with larger velocity than the ATP-binding deficient mutant, which differs from our model calculations. We consider that our model dynein of the ATP-hydrolysis deficient mutant detaches from MT quickly so that it is difficult to complete ATP cycles in many trajectories, which makes the velocity estimate difficult.

500

501 **The bipedal mechanism: Pathway analysis**

So far, we described overall behavior of motility in our kinetic model, showing that the model can reproduce many of previous experimental observations. Now, we analyze the underlying mechanisms emerged from our model. For this purpose, we focus on the case of $[ATP] = 1 \text{ mM}$ with no external force, unless otherwise denoted.

Fig 4 shows the whole network of the states-to-state transition dynamics, which, albeit of high complexity, contains full of mechanistic information. Hereafter, we decipher this complex network. Of the 75 states, the network contains only those that appeared in our 10000 trajectories.

510

Fig 4: The whole network of pathways. Black filled and open circles represent the dimeric dynein states bound on and detached from MT (the dead-end), respectively. Red (blue) arrows indicate the transitions where the state in the red (blue) monomer changes, whereas the green arrows mean the diffusive motions along MT. Integers written on the arrows represent the number of transition times observed in simulations, with which the thickness of the arrow correlates. For the meaning of the label of each state, see the text. 1DMDM+, 26DMDM 0, and 51751DMDM - are marked with stars, to emphasize their high populations.

518

519

Of the 75 states, the most populated states were the 26DMDM0 state (see S2 Table for the list of high population states); both monomers are in the DM states located at the same position along MT (implicitly, bound on different proto-filaments) (a star mark located near the center of the figure).

Starting from this ground state 26DMDM0 and following probable transitions (thick arrows, with the counts larger than 5000), we find a pair of probable cyclic pathways. The one that makes a clockwise rotation in the upper side of the figure is

Path 1: 26DMDM0 → 31DMT0 → 36DMT*0 → 41DMD*M0 → 51DMDM- → 55DDM- → 30DDM0 → 26DMDM0

On this pathway, from the 26DMDM0 state to the 51DMDM- state via three transient states, the blue motor domain completed one ATP hydrolysis cycle while the red one remained in the DM state, by which the blue motor domain proceeded by one step forward via recovery-stroke and the subsequent power-stroke of the linker (Remember that, to distinguish two identical motor domains, we call the first and the second domains as the red and the blue domains, respectively). The 51DMDM- state is a long-lived intermediate state (star-marked in the figure). Subsequently, the lagging red motor domain detached from MT reaching to 55DDM-, which is followed by the diffusive motion of the red motor domain 30DDM0. Finally, the red domain rebound to the MT returning into the starting state 26DMDM0. When the dimeric dynein repeats this cycle more than once, this process is normally called the inchworm motion.

Notably, during this cycle, the ATP hydrolysis reaction occurred only in the leading (blue) motor domain, whereas the lagging (red) motor domain is simply dragged by the leading one. This is in harmony with the observation that the mutants that impair motor activity still move in one direction. The other prominent cycle (a clockwise cycle in the bottom side of the figure), which is related to the first one by symmetry, is,

Path 1': 26DMDM0 → 27TDM0 → 28T*DM0 → 29D*MDM0 → 1DMDM+ → 21DMD+ → 46DMD0 → 26DMDM0

in which the red motor domain hydrolyzes one ATP to drive the system forward. Clearly, the mechanism is identical to the first cycle.

Thus, while the pathway analysis exhibit extremely diverse routes, we find a dominant cyclic pathway which corresponds to the inchworm motion.

Distinct bipedal motions

While we found one prominent cyclic pathway, Path 1, in the previous section, due to the complexity of the whole network in Fig 4, we need a more systematic analysis to reveal various pathways. Given that each motor domain has the highest population in the DM state (S2 Table), we systematically seek pathways that connect the dimeric dynein states where both motor domains take the DM states. Among several possible combinations, we found the two cases are dominant; 1) the one starting from and ending to the 26DMDM0 state and 2) the other starting

from 1DMDM+ and ending at 51DMDM- without passing through the 26DMDM0 state. We note that, by symmetry, we also observed the case from 51DMDM- to 1DMDM+, of which the mechanisms are identical to the second case.

We depict the schematic pictures of the major cyclic paths starting from and ending at the 26DMDM0 state in Fig 5A. Clearly, all these correspond to the inchworm motions when the cycles are repeated more than once. In the 10000 trajectories, we identified 15896 steps of inchworm motions. The figure contains, in the second row, the Path 1 already described above based on the visual inspection.

Path 1: 26DMDM0 $\rightarrow$ 31DMT0 $\rightarrow$ 36DMT*0 $\rightarrow$ 41DMD*M0 $\rightarrow$ 51DMDM- $\rightarrow$ 55DDM- $\rightarrow$ 30DDM0 $\rightarrow$ 26DMDM0

Fig 5: The two distinct bipedal working pathways. (A) The inchworm-like pathway. (B) The hand-over-hand pathway. Schematic diagrams and the whole network are drawn on the left and the right, respectively.

As described above, the leading motor domain made one ATP hydrolysis cycle driving this domain forward by one step, whereas the lagging motor domain moved via diffusive motions dragged by the leading domain. In addition, we find a branch path depicted in the top row of the figure, in which the detached leading motor domain diffused backward, which is followed by the recovery-stroke and subsequently the power-stroke. Overall, the molecule returned to its original state and position. Moreover, we find another branch path depicted in the bottom line of the figure, where the detached blue motor domain diffused forward. The following steps include recovery-stroke and then the power-stroke in the blue motor domain. Together, the dynein moved 2 steps forward, one by diffusion and the other by the power stroke. Thus, while a pair of linker recovery-stroke and the subsequent power-stroke in the leading domain contributes to the one forward step, additional diffusive motions, if any, can modulate the motions.

Next, we consider the paths starting from 1DMDM+ and ending at 51DMDM- without passing through the 26DMDM0 state, of which a schematic picture is drawn in Fig 5B. Albeit high diversity, these paths all correspond to the hand-over-hand motions. In the 10000 trajectories, we identified 3328 steps of the hand-over-hand motions, which is about one fifth of the inchworm motions (Fig 2F). This route contains many branches, but each path includes one or more relatively slow transition processes, making this entire route not as frequently used as the Path 1.

591 Among the many branches during the hand-over-hand motion, the most frequently used path
592 was as follows,

593 Path 2: 1DMDM+ $\rightarrow$ 6DMT+ $\rightarrow$ 11DMT*+ $\rightarrow$ 36DMT*0 $\rightarrow$ 41DMD*M0 $\rightarrow$ 51DMDM-
594 During the process, fueled by the ATP hydrolysis reaction cycle in the blue motor domain, the
595 blue domain moved from the rear side of the red motor domain to the forward side by 2 steps,
596 and thus 16 nm. We note that one of the 2 steps is via diffusive movement. More in details,
597 starting from the 1DMDM+ state, the internal tension from the forward red motor domain in-
598 duces the ADP dissociation/ ATP binding in the rear blue domain, leading to the detachment
599 from the MT (6DMT+) and subsequently recovery stroke (36DMT*+). Then, the lagging blue
600 domain diffuses forward (36DMT*0), which is followed by the ATP hydrolysis (41DMD*M0).
601 Finally, the power-stroke in the blue domain moved the blue domain one more forward step. We
602 note that in this process the one (blue) motor domain moved by two steps, one via diffusion and
603 the other by the power-stroke. The other (red) motor domain remained in the DM state through-
604 out. Interestingly, the hand-over-hand motion in the model dynein is qualitatively different from
605 that of kinesin-1 (or conventional kinesin), the neck-linker docking (classically termed the
606 power-stroke) occurs in the MT-bound leading head. Whereas, our model dynein uses the
607 power-stroke of the lagging motor domain to move itself forward. When the dimeric dynein
608 continues on this bipedal motion, the next step is that the red motor domain moves forward by
609 two steps using ATP hydrolysis reaction. Therefore, mutants that impair one motor domain ac-
610 tivity cannot move by this mechanism. There exist similar paths closely related to the Path 2,
611 such as

612 Path 2': 1DMDM+ $\rightarrow$ 6DMT+ $\rightarrow$ 31DMT0 $\rightarrow$ 36DMT*0 $\rightarrow$ 41DMD*M0 $\rightarrow$ 51DMDM-
613 which differs from the Path 2 only in the third state 31DMT0. These are essentially the same.

614 Occasionally, we observed that both the blue and red motor domains made diffusional move-
615 ment during one ATP cycle in the blue domain. The path can be described as

616 Path 3: 1DMDM+ $\rightarrow$ 6DMT+ $\rightarrow$ 31DMT0 $\rightarrow$ 56DMT- $\rightarrow$ 61DMT*- $\rightarrow$ 66DMD*M- $\rightarrow$
617 70DD*M- $\rightarrow$ 45DD*M0 $\rightarrow$ 55DDM- $\rightarrow$ 51DMDM-

618 This whole path contains three diffusive movements, in addition to one ATP hydrolysis cycle in
619 the blue motor domain.

620 In summary, starting from the states where both motor domains are in the most stable DM
621 states, we found two prominent cyclic pathways, Paths 1 and 2, which correspond to the inch-
622 worm and the hand-over-hand motions, respectively. By counting the respective cycles in the
623 trajectories, we found that the inchworm motions are more probable than the hand-over-hand

624 motions for all the [ATP] and the external force conditions tested here (Fig 2F). The predomi-
625 nance of the inchworm steps in the model dynein is in harmony with the experiment data [10].
626

627 **Fast-track, slow-track, and back-step**

628 So far we described the inchworm and the hand-over-hand motions in the model dynein step-
629 ping, but the whole network includes many more sub-dominant pathways. To address effects of
630 dynein stepping pathway in its velocity, we classified fragments of trajectories by the short-term
631 velocity defined within the fragment. Here, we define the three classes, fast-track, slow-track,
632 and backward move and discuss dominant pathways in each class (we exclude the medium-ve-
633 locity class because it corresponds to the dominant pathways discussed above). The fast-track is
634 defined as the fragment of trajectories that have their velocities faster than the third quartile
635 value (480 nm/s). Similarly, the slow-track is defined as those with the velocities slower than
636 the first quartile value and that are positive (54 nm/s). The backward move is defined as the
637 negative velocities in the fragment of trajectories.

638 First, Fig 6A depicts the fast-track pathways and its schematic mechanism. One prominent
639 path starts from the 1DMDM+ state (or equivalently 51DMDM- state) and proceed via the Path
640 (the hand-over-hand manner).

641 Path 2: 1DMDM+ $\rightarrow$ 6DMT+ $\rightarrow$ 11DMT*+ $\rightarrow$ 36DMT*0 $\rightarrow$ 41DMD*M0 $\rightarrow$ 51DMDM-
642

643 **Fig 6.** Dominant motions in different velocity regimes. (A) Fast-track motion. (B) Slow-track
644 motion. (C) Backward motion. Schematic diagrams and the whole network are drawn on the left
645 and the right, respectively.

646

647 As above, in the fast-track pathways, there exist some small variant paths. For example, when
648 the order of the recovery stroke and the diffusion is exchanged from the above path, we obtain a
649 variant path depicted at the top line in the left side of Fig 6A. Alternatively, starting from the
650 initial 1DMDM+ state, detachment and the diffusion of the rear domain can precede the nucleo-
651 tide exchange reaction, which is drawn at the bottom line of the left side of Fig 6A. In summary,
652 the hand-over-hand motion dominates the fast movement.

653 Next, we discuss the slow-track, of which schematic pathways are drawn in Fig 6B. The
654 prominent pathway observed is

655 Path 4: 26DMDM0 $\rightarrow$ 31DMT0 $\rightarrow$ 36DMT*0 $\rightarrow$ 11DMT*+ $\rightarrow$ 16DMD*M+ $\rightarrow$
656 26DMDM0

The first three states in this path are identical to that in the Path 1. From the 36DMT*0 state, the blue motor domain diffused backward (11DMT*+). Then, the blue domain was bound on the MT upon ATP hydrolysis (16DMD*M+), which is followed by the power-stroke to return the 26DMDM0 state. This path was illustrated in Fig 2A at around the time 0.70 - 0.74 s where we see long pause with an instantaneous back step of the blue domain. Overall, the molecule did not proceed forward, but came back to its original location. A variant of the Path 4 is also depicted at the bottom line of the left side of Fig. 6B. Pathways observed in the low velocity class always include a backward diffusion.

Finally, we describe the case in which the dimeric dynein moved backward. Fig 6C merged with Fig 6B represent a pathway,

Path 5: 51DMDM- $\rightarrow$ 56DMT- $\rightarrow$ 6DMT+ $\rightarrow$ 11DMT*+ $\rightarrow$ 16DMD*M+ $\rightarrow$ 26DMDM0

The last three states in this path are identical to those in Path 4. A crucial feature in this pathway is that, in the very first transition, the leading blue domain detached from MT, which is in contrast to the detachment of the lagging domain in the Path 2; the hand-over-hand motion. Once the leading blue domain is detached, it cannot diffuse forward, but can diffuse backward, due to the restraint from the MT-bound red motor domain. In the Path 5, the blue domain diffused backward by two steps, which is followed by the recovery stroke and then the power-stroke motion in the blue domain. Overall, the dimeric dynein moved backward by one step.

Discussion

The observed primary inchworm motion uses one ATP per a dimer step

Here, we propose the kinetic model, which can reproduce extremely versatile modes of dynein movement. Our model suggests that the dominant pathway is the inchworm motion, which is about 5 times as many as the hand-over-hand motions. This is consistent with a recent report that classifies the modes of movements of dynein finding that about 80% of steps are of the inchworm type motions [10].

Notably, among some variants, the most prominent inchworm pathway in our model dynein, Path 1: 26DMDM0 $\rightarrow$ 31DMT0 $\rightarrow$ 36DMT*0 $\rightarrow$ 41DMD*M0 $\rightarrow$ 51DMDM- $\rightarrow$ 55DDM- $\rightarrow$ 30DDM0 $\rightarrow$ 26DMDM0

uses only one ATP hydrolysis per a dimer step. Namely, only the leading motor domain moves via ATP-dependent linker power-stroke coupled with a change in the stalk angle, whereas the lagging motor domain is moved via diffusion dragged by the leading motor domain without the ATP hydrolysis cycle. In this sense, the observed model is clearly different from previously

suggested inchworm models where two ATP hydrolysis are assumed to occur per a dimer step (e.g., Fig. 4 in [51], Fig. 2 in [2]). It should be noted that the current model do include such a pathway. In Fig. 3, we find the transition from 51DMDM- to 52TDM-, which continues to make the following cycle,

Classic inchworm path: $26\text{DMDM}0 \rightarrow 31\text{DMT}0 \rightarrow 36\text{DMT}^*0 \rightarrow 41\text{DMD}^*\text{M}0 \rightarrow 51\text{DMDM}- \rightarrow 52\text{TDM}- \rightarrow 53\text{T}^*\text{DM}- \rightarrow 54\text{DM}^*\text{DM}- \rightarrow 26\text{DMDM}0$

where each motor domain hydrolyzes one ATP, resulting in two ATP consumption per a dimer step. In our model, this pathway is minor since the transition from 51DMDM- to 52TDM- occurs with a relatively low probability. A recent single-molecule assay measured the AAA+ ring angles relative to MT, finding that about 50% of motor domain steps are coupled with small angle changes in the AAA+ ring [52]. The Path 1 is perfectly in harmony with this result because only the leading motor domain takes the ATP hydrolysis cycle which exerts the force and leads to changes in the AAA+ ring angle. On the other hand, the classic inchworm path is not compatible with this single-molecule assay data because it contains twice of ATP hydrolysis cycles and steps and thus it takes 100% coupling between the stepping and the angle change.

Within our model, we assume that the stalk lean towards the MT axis by about 15 degree in the pre-power-stroke state, T* and D*M states, relative to the post-power-stroke state. However, the lifetimes of the pre-power-stroke states are rather short: From the populations in every states (S2 Table), we estimated the probability to have at least one pre-power-stroke state is only 15%, as a whole. Thus, due to short lifetimes of the pre-power-stroke states, some of these angle changes in the stalk might not been observed by the single-molecule assay [53].

It is interesting to discuss an analogy to other molecular motors that are known to use the inchworm motions: Some helicases and ATP-dependent chromatin remodelers are known to use the inchworm motions to proceed along DNA [54–56]. The inchworm motions in these molecules are realized by two subdomains where the ATP hydrolysis is catalyzed at the interface of the two subdomains. In the apo-state, the subdomains are bound on DNA in an open form. Upon ATP binding, subdomains close by sliding one of the two subdomains on DNA by one base-pair. After ATP hydrolysis, the subdomains return to the open form by moving the other subdomain on DNA by one base-pair, which results in the one base-pair inchworm motion. Notably, one ATP hydrolysis cycle is sufficient to achieve one inchworm motion in these cases. Thus, this usage of ATP is similar to the inchworm motions found in the current simulations for the model dynein.

723

The observed hand-over-hand motion agrees with the previous models

We observed the hand-over-hand motions as a sub-dominant pathway. Even though there exist many branch paths, a prominent one is

Path 2: $1DMDM^+ \rightarrow 6DMT^+ \rightarrow 11DMT^* \rightarrow 36DMT^*0 \rightarrow 41DMD^*M0 \rightarrow 51DMDM-$ which is actually identical to the hypothetical model suggested previously [11,53]. In our kinetic model, however, this mode is sub-dominant.

It is interesting to note that the hand-over-hand motion in the dynein is distinct from that in kinesin. In kinesin, chemical events in both heads are more tightly coordinated. Starting from the two-head bound states, the ATP hydrolysis in the lagging head reduces its binding affinity to MT and results in the dissociation from the MT. The ATP-dependent neck-linker docking (classically called neck-linker power-stroke) occurs in the originally leading head. On the other hand, the hand-over-hand motion found in our model dynein contains the full of ATP-hydrolysis cycle in the originally lagging motor domain, while the originally leading domain stays in the ADP-bound state. The ATP-dependent power-stroke occurs in the originally lagging motor domain.

Bipedal motion under external force

It has been suggested that a load exerted by bound cargos speeds up dynein movements by activating some conformational changes. However, our model propose a possibility that this increase in the velocity under load is an intrinsic feature of the dynein motor domain. As in Fig 2D, the velocity slightly increased with a weak external force; e.g., at $[ATP] = 1 \text{ mM}$, the velocity with 0.5 pN external force was larger than that without external force. This slight increase in the velocity under a weak opposing force was also reported in previous experimental studies [40,57]. The load-dependent cancellation of dynein auto-inhibition has been proposed for the cause of the phenomenon, however, the molecular basis for such cancellation was still obscure. Our model analysis may partly explain the reason for acceleration of dynein motility under a small backward load.

In order to understand the underlying mechanism of this increase, we sought the difference in the paths with and without 0.5 pN of external force. We extracted fragments of paths that are significantly more frequent with the 0.5 pN force compared to the case of no force (Path 6, depicted in Fig 7)

Path 6: $2TDM^+ \rightarrow 3T^*DM^+ \rightarrow 4D^*MDM^+ \rightarrow 24D^*MD^+ \rightarrow 49D^*MD0 \rightarrow 74D^*MD- \rightarrow 46DMD0 \rightarrow 26DMDM0$

Fig 7: Paths observed in the case of 0.5pN external force more frequently than the case of no external force. Schematic diagrams and the whole network are drawn on the left and the right, respectively.

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In the first half of the path 6, the leading red motor domain takes the ATP hydrolysis reaction, which inevitably peels out the lagging motor domain from MT due to the internal force. We then ask why this path was enhanced by the 0.5pN force. First, total incoming flows to 2TDM+ were equally probable with and without the external force. Second, the transition from 2TDM+ to 3T*DM+ was notably enhanced by the external force. Without the external force, the 2TDM+ state tends to make a transition to 2TDM0 via backward diffusion of the red domain. The external force affects this route in two mutually opposing mechanisms: 1) The backward diffusion is accelerated by the external force as in eq. (5), which increases the transition to 2TDM0. 2) The MT-bound blue motor domain receives the half of external force in 2TDM0 state, but not in 2TDM+ state, which suggests that the 2TDM0 has extra energy cost due to external-force based factor $f_{ext}(F/2) = 10^{(F/2)/pN}$, which thus reduces the transition to 2TDM0. In fact, the two effects are nearly cancelled out, but, in our current estimate, the second effect is slightly larger. This inhibitory effect to 2TDM0 explains the slight increase in the transition frequency to 3T*DM+. We, however, note that our estimate in the second effect comes from the experimental data of detachment force for *Dictyostelium* dynein, which contain some uncertainty. If the detachment force were 3 pN, for example, instead of 2 pN used here, the second effect would be weaker than the first effect, and thus we may not see the slight increase in the velocity upon a weak external force.

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Effects of internal force and diffusion rates

Our model contains some parameters of which appropriate values are hardly known from experiments to date. Here, we address effects of two such parameter values; the internal force factor, f_{int} , and the diffusion rates, $\lambda = \lambda_{for} = \lambda_{back}$ without the external force. As the default, we set $f_{int} = 10000$ and $\lambda = 200$. Here, we repeated the same simulations with three other parameter choices and compared them with the default one (S3 Fig). (1) $f_{int} = 10000$ and $\lambda = 200$ (the default set), (2) $f_{int} = 1$ and $\lambda = 200$, (3) $f_{int} = 10000$ and $\lambda = 500$, and (4) $f_{int} = 10000$ and $\lambda = 1000$. We plot the results for run-lengths (S3A and S3B Fig) and velocities (S3C and S3D Fig) as functions of [ATP] (S3A and S3C Fig) and the external force (S3B and S3D Fig). The [ATP]

dependence was tested with no external force. The external force dependence was investigated with $[ATP] = 1 \text{ mM}$.

At first, the two major features were kept for all the cases. 1) The model dynein does not move uni-directionally without ATP. 2) The run-length decreased as $[ATP]$ increased.

Looking into the effect of the diffusion rate λ , we find that all the results for the cases (1), (3), and (4) are rather similar each other, suggesting that the diffusion rate value does not affect the overall behavior significantly.

When we removed the effect of internal force f_{int} in (2) (green curves in S3 Fig), the run-length at higher $[ATP]$ becomes longer, compared to the default set (1) that contains internal force (S3A Fig), while the velocity is significantly lower than the default case (S3C Fig). Thus, the system that lacks the internal force effect tends to bind more strongly to the MT, making the run-length longer and the velocity lower. The effect of the internal force, f_{int} , is to accelerate the dissociation of the lagging motor domain ($DM \rightarrow D$), which facilitates the forward step of that domain increasing the velocity in one hand, and enhances the detachment of the entire dynein from MT thus decreasing the run length in the other hand.

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Next, we examined the walking mechanisms in different f_{int} and λ setups (S3E Fig). It is clear that in the system without the internal force $f = 1$, the hand-over-hand moves become more prominent, while the inchworm moves are less probable, compared to the default case with the internal force. This is because the pathway; $1DMDM^+ \rightarrow 21DMD^+ \rightarrow 46DMD0 \rightarrow 26DMDM0$, observed frequently in the default case $f = 10000$ is less prominent for the case of $f = 1$, due to the deceleration of the first step in this pathway. In the transition $1DMDM^+ \rightarrow 21DMD^+$, the dissociation of the lagging motor domain is facilitated by the presence of the internal force. At the same time, when the lagging motor domain detaches from MT, it binds ATP leading to the hand-over-hand motions. Therefore, the internal force is important for the characteristic walking manner, inchworm motion.

Additionally, we also notice larger diffusion constant value shows slight increase in the inchworm motions. This is because the motor domain often moves diffusively twice within one ATP cycle in the case of large λ value, and so the lagging motor domain tends to detach via internal force and moves to the forward direction. When it then binds to MT, this pathway goes to $26DMDM0$, regarded as the inchworm motions.

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822 Limitation and future prospect

As discussed, our kinetic model includes some parameters which are not derived from experiments or which are taken from experiments with mixed conditions such as different species or different ionic strengths. In particular, data on the effect of external force were missing for *Dictyostelium* dynein due to inherently weaker MT binding affinity of *Dictyostelium* dynein than those from yeast or human. However, since the time courses of movements have been measured, we could infer these model parameters via Bayesian approaches, which is kept for future studies.

In addition, for simplicity, we limit ourselves the relative positions of the two motor domains being +8 nm, 0 nm, or -8 nm. It is straightforward to extend it to 16 nm or 24 nm to make it closer to experimental estimates. Limiting the relative position to discrete states is perhaps not an ideal setup. Extending it to continuous value would be desired. Moreover, it has been known that dynein occasionally make sidesteps to different protofilaments, which, for simplicity, we have not included in the model.

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838 Conclusion

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We proposed a kinetic model for bipedal motions of cytoplasmic dynein, simulated it via the Gillespie Monte Carlo method obtaining results consistent with many of previous motility experiments. The detailed pathway analysis provided new and versatile molecular mechanisms of bipedal motions of dynein, including the inchworm motions and the hand-over-hand motions. The kinetic model contains the 5 states of each motor domain based on the ATP-dependent structural changes in the linker and the MTBD, as well as 3 states for relative position of the two motor domain, resulting into $5 \times 5 \times 3 = 75$ states together. The master equation in this 75 states was solved by the Gillespie algorithm. As a result, with a single parameter set, we successfully reproduced many characteristic behavior of dynein found in previous experiments; the inchworm motions as the dominant mode, the hand-over-hand motions, backsteps, and stagnation. Our model suggests that, in the prominent inchworm movement, only the leading motor domain moves via the ATP-dependent linker power-stroke motions, while the lagging motor domain is moved diffusively dragged by the leading domain. In addition, the hand-over-hand motion in the model dynein is distinct from that of kinesin by the usage of the power-stroke.

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857 **Supporting Information**

858

859Supporting Figure Caption

860**Fig S1: Effects of internal forces.** The model takes into account the asymmetric detachment
861rates of the motor domain; when both domains are bound on MT, the lagging domain dissoci-
862ates more rapidly than the leading domain. In the master equation approach, the effect is real-
863ized by introducing a multiplying/dividing factor, f_{int} in the transition rates, which implies the
864changes in the free energy by $\ln(f_{\text{int}})$. Taking care of the detailed balance, we need to introduce
865the same factor in many transitions connected in the kinetic network. The introduced multiply-
866ing/dividing factors are defined in the figure.

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868**Fig S2: All the 10000 trajectories superimposed for various [ATP] and external forces.**

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870**Fig S3:** Motility with different model parameters are examined.

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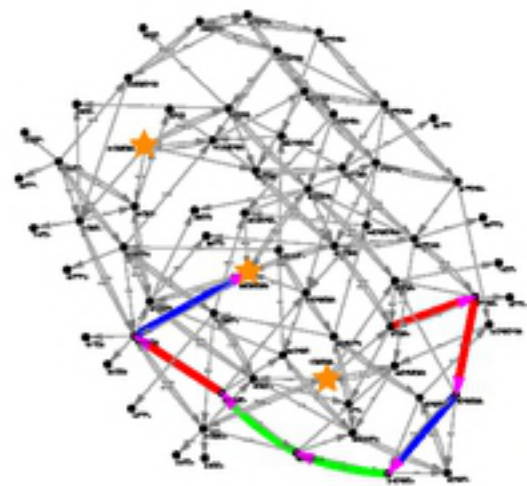
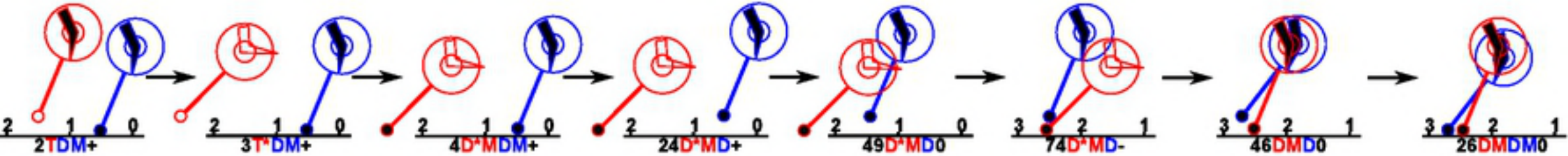
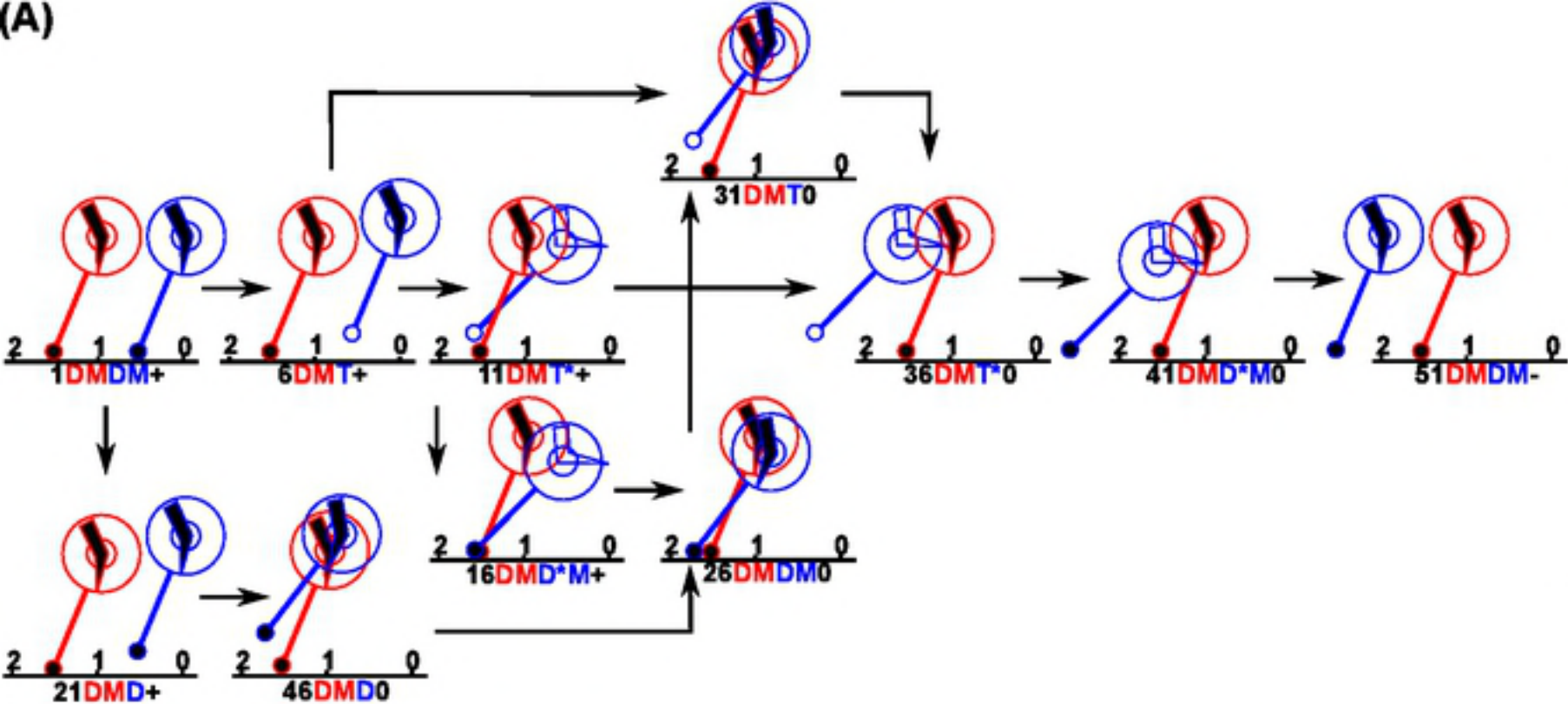
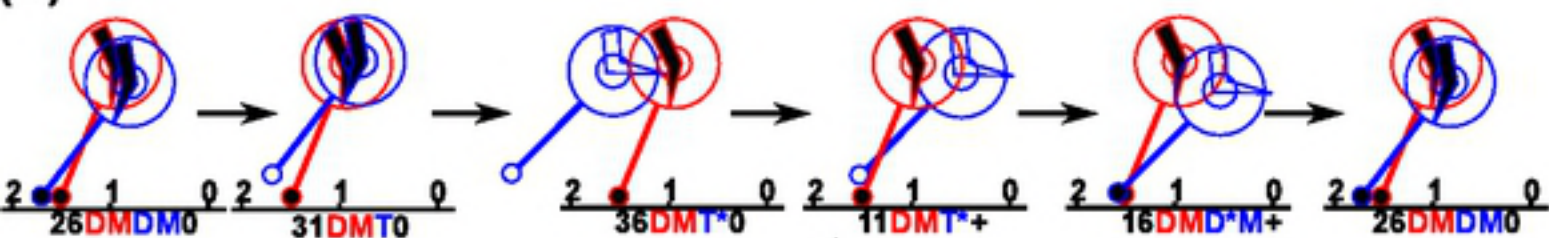


figure7

(A)



(B)



(C)

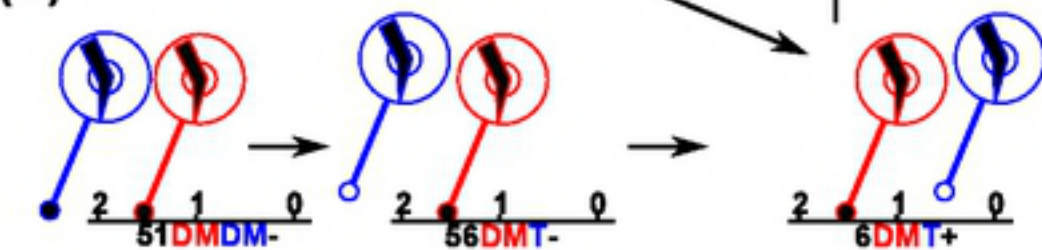
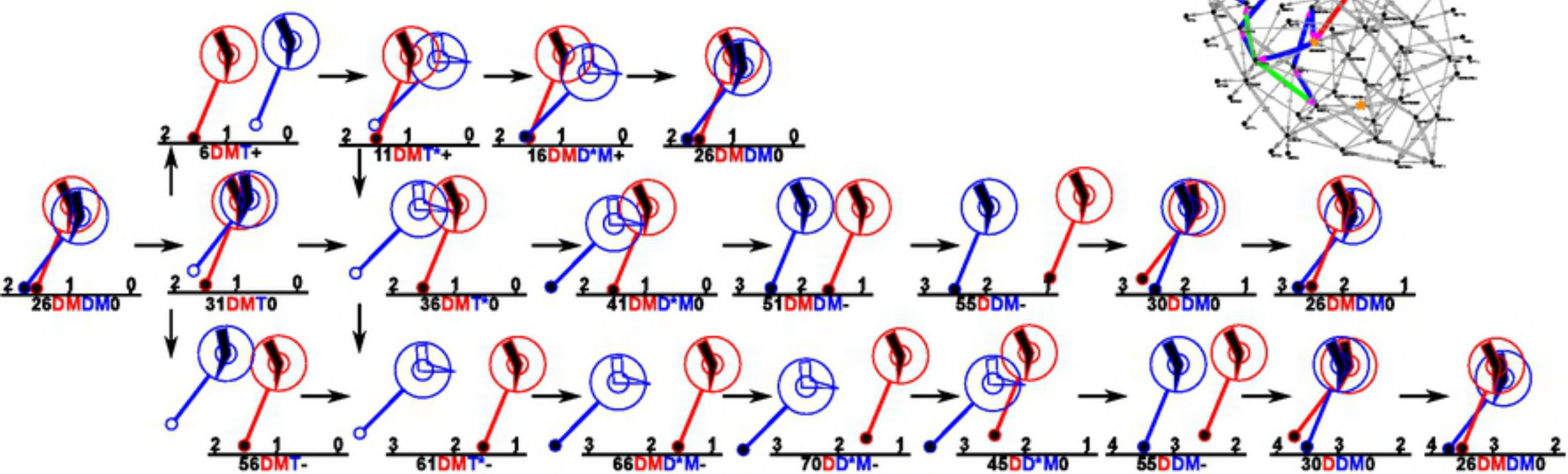


figure6

(A)



(B)

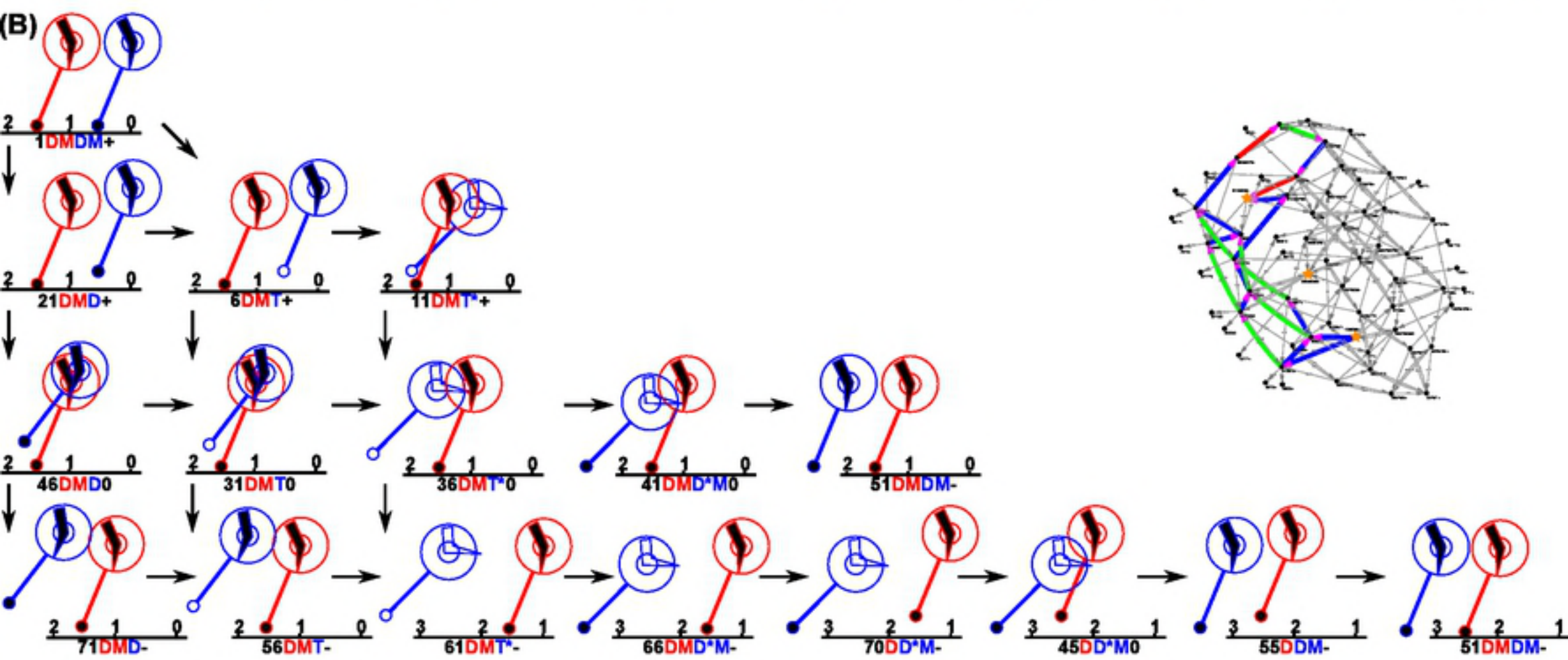


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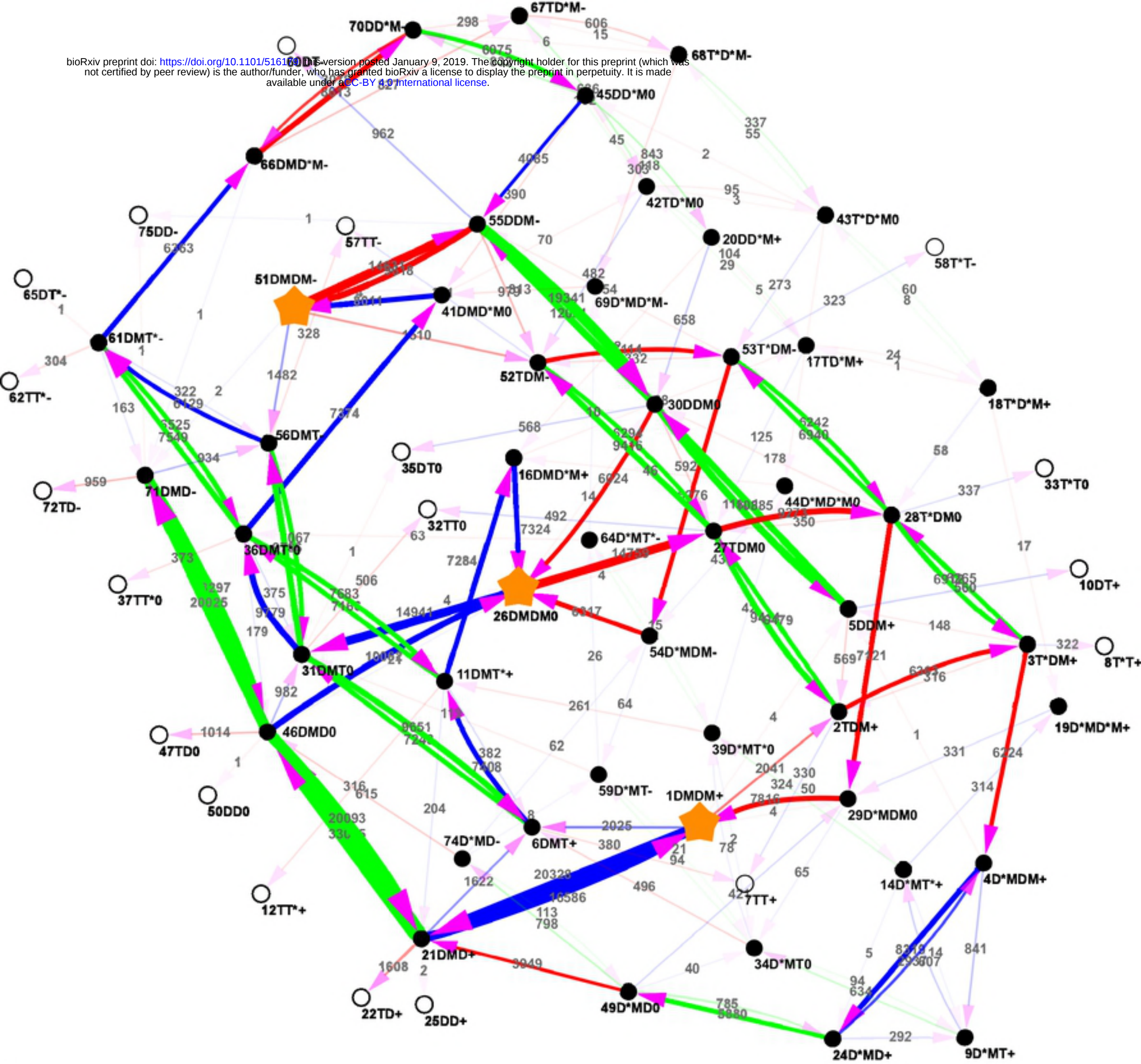


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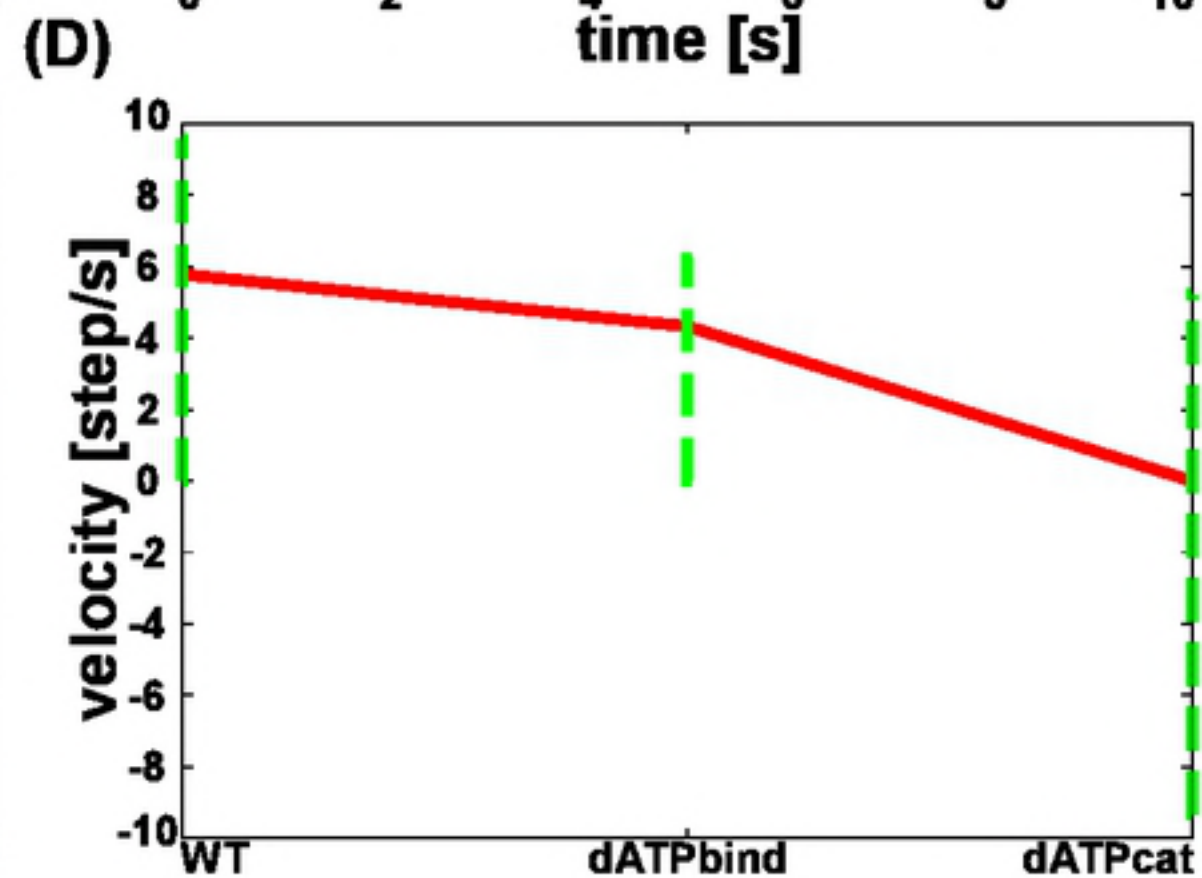
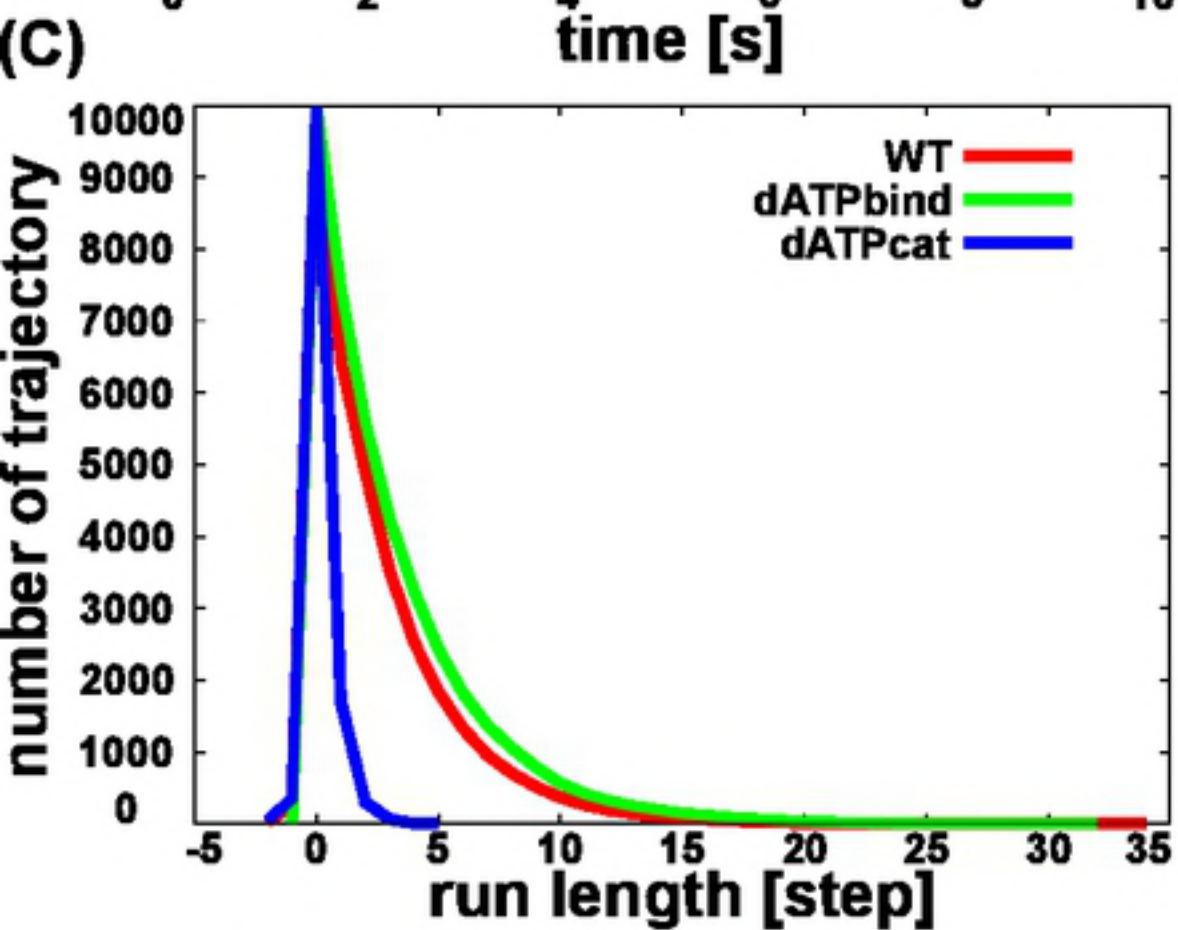
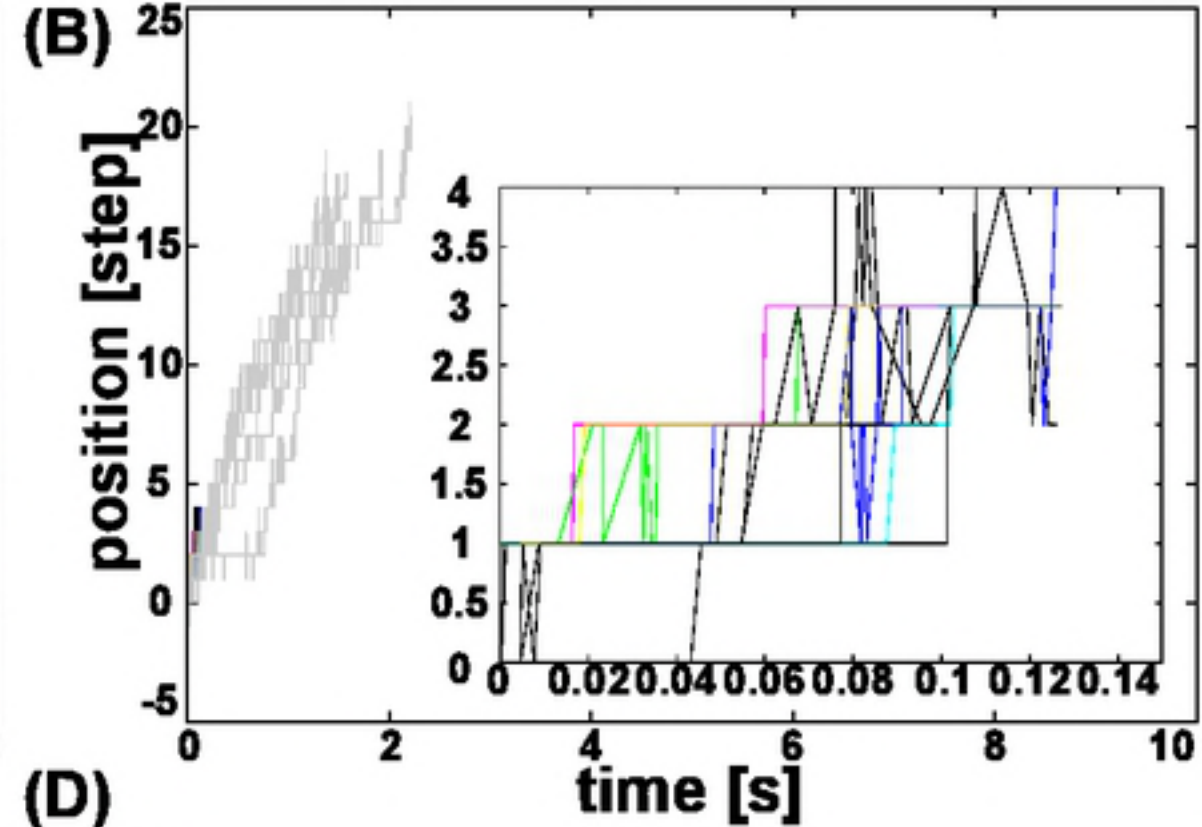
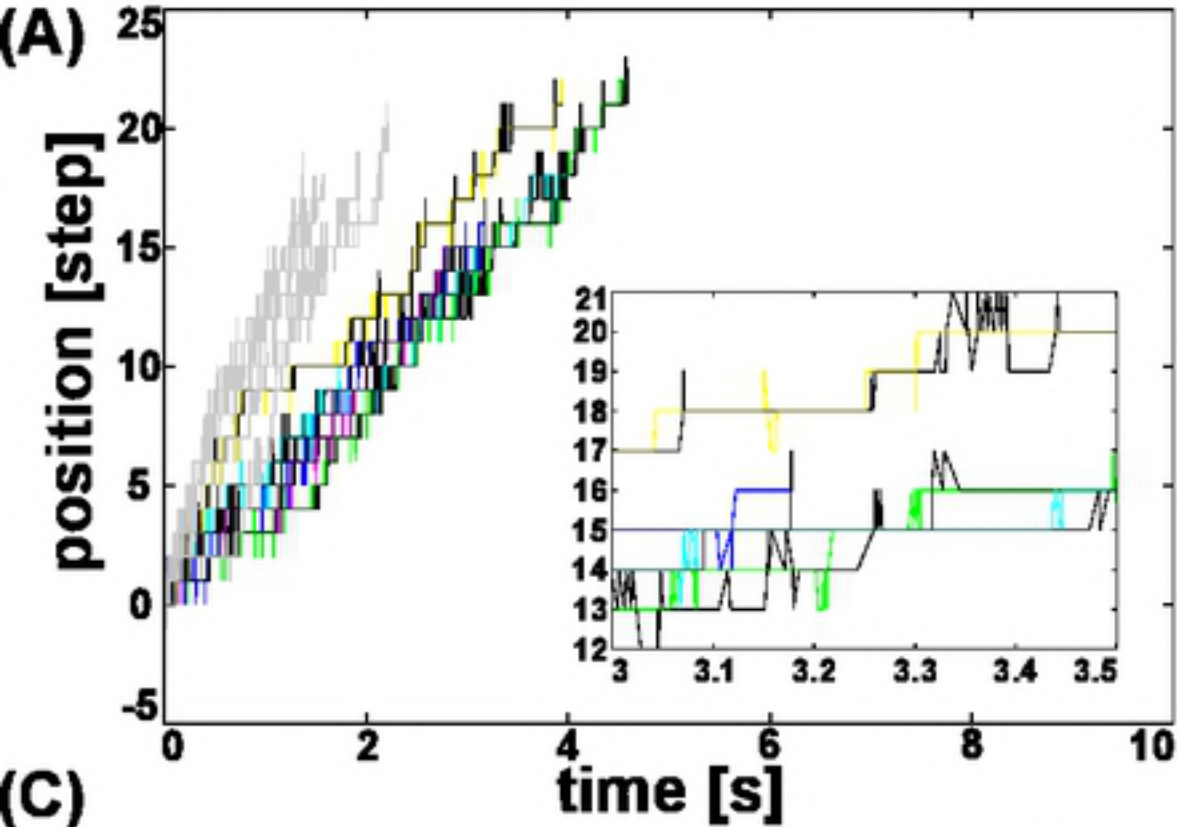


figure3

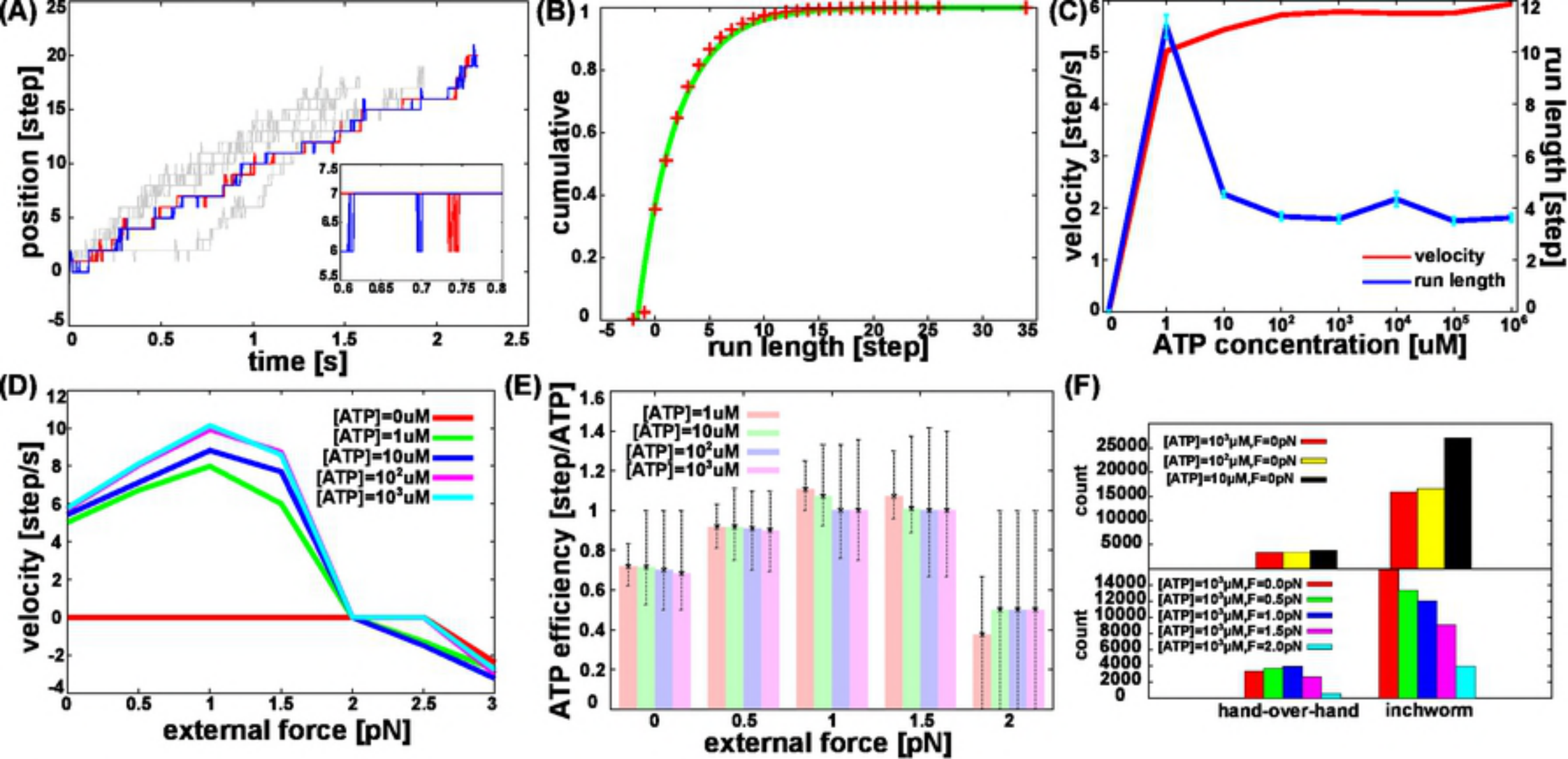


figure2

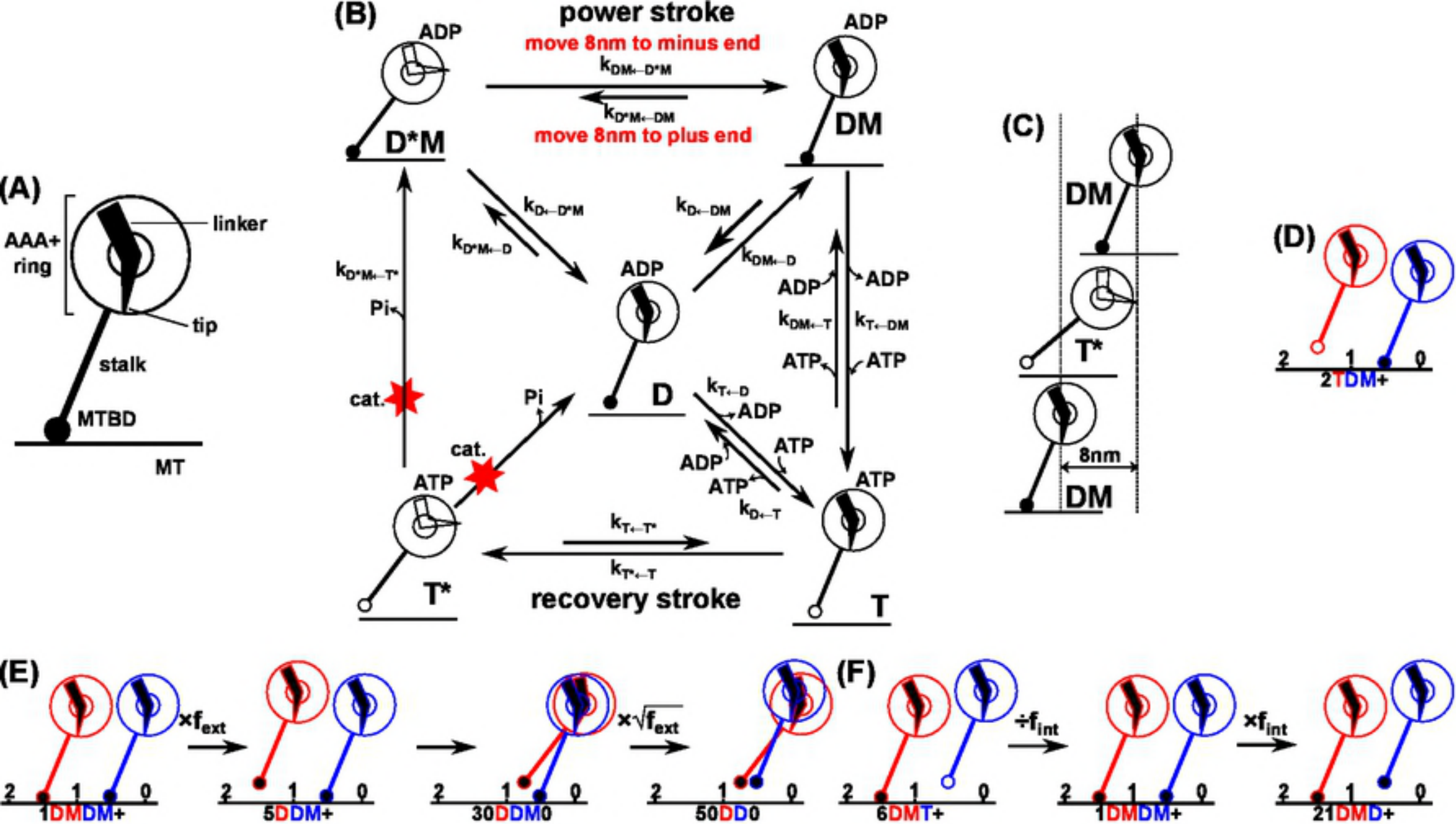


figure1