Supporting Material for

The Winch Model Can Explain Both Coordinated and Uncoordinated Stepping of Cytoplasmic Dynein

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Supporting Methods

Elastomechanical Model

A dimeric molecule can be found in one of

$$N = 5^2 \times (1 + 2 + r) \tag{S1}$$

states, where 5 is number of chemical states of each head (ATP, ADP.Pi, ADP*, ADP or no nucleotide) and the dimer can either be detached from MT, bound with one head, or bound with both heads with r possible relative positions. For $r = 34$ this gives $N = 925$ states. At a given load force the energy of each state can be calculated from Eq. (3) and contains contributions from elastic deformations of both stalks, interaction between the heads, as well as work performed against the load. We obtained the configuration in each state by energy minimization.
Each stalk is described as an ideal elastic beam with bending and torsional persistence and torsional lengths λ_p and λ_c. The elastic energy of its three-dimensional deformation is

$$U_{el} = \frac{1}{2} k_B T \left\{ \lambda_p \int_0^L \left[\left(\frac{\mathrm{d}\theta}{\mathrm{d}l} \right)^2 + \sin^2\theta \left(\frac{\mathrm{d}\varphi}{\mathrm{d}l} \right)^2 \right] \mathrm{d}l + \lambda_c \int_0^L \left(\frac{\mathrm{d}\psi}{\mathrm{d}l} + \cos\theta \frac{\mathrm{d}\varphi}{\mathrm{d}l} \right)^2 \mathrm{d}l \right\}. \tag{S2}$$

Here $k_B T$ denotes the thermal energy, θ, φ, ψ the three Euler angles, describing the local deformation of the stalk, and l the arc length of the stalk. We discretized each stalk shape with 20 segments whose three Euler angles were subject to variation. The minimization was performed by a custom made C++ program based on a Monte Carlo method. The initial configuration was chosen with straight stalks, unless such a state violated the hard core repulsion between the heads or stalks (if this was the case the stalks were additionally inclined for the initialization). At each Monte Carlo step a candidate configuration was generated by randomly varying the direction of one stalk segment and accepting the move if there was no overlapping between the rings and if it led to a lower elastic energy.

Kinetic Model

We simulated the stepping of dynein with a custom made C++ program based on a kinetic Monte Carlo method (Gillespie algorithm). The rates of allowed transitions are listed in Table 3. For transitions that involve conformational changes, the rates were multiplied with the corresponding Boltzmann factors, obtained from the energy difference between the states. Each run was simulated until the dimer dissociated from the MT. The stepping statistics were obtained from the analysis of 5000 simulated runs. When obtaining the average run length only those with run lengths exceeding 10 nm have been considered.

Supporting Figures

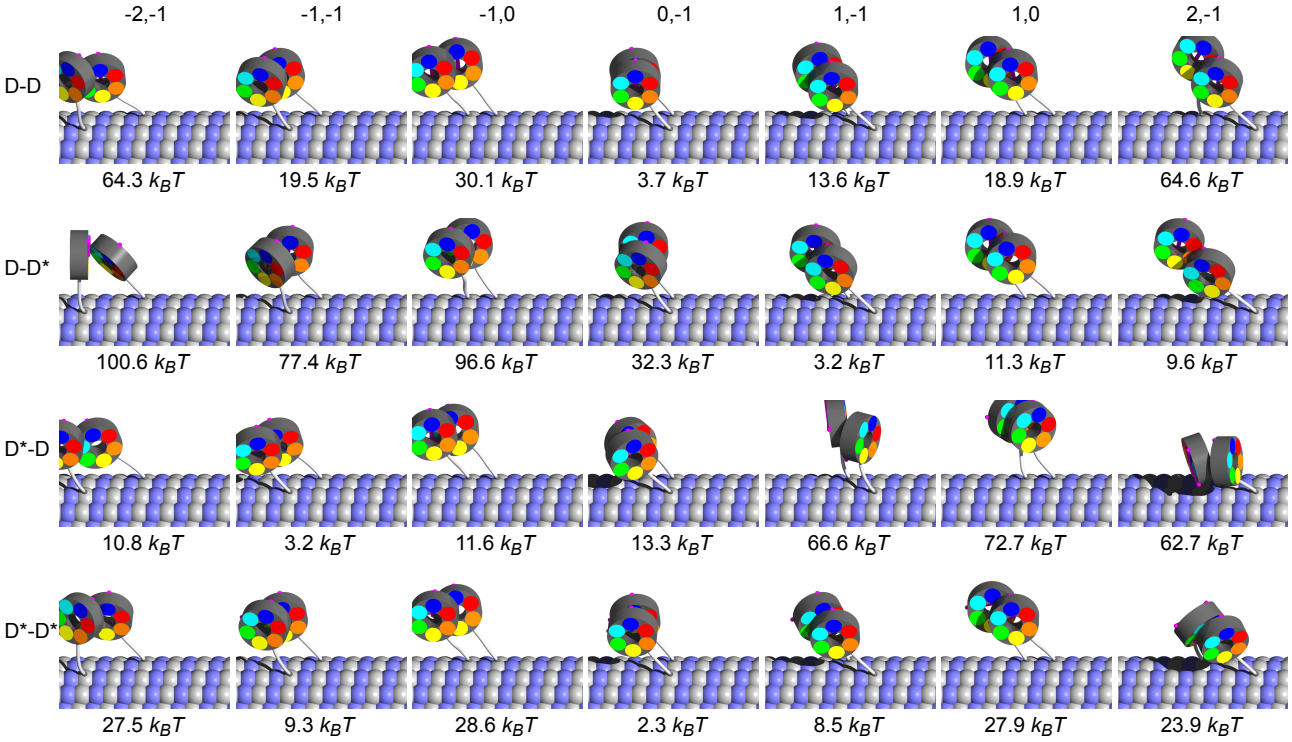


Figure S1: A selection of minimum energy configurations of the tightly coupled dimeric dynein molecule. Each line contains a combination of linker positions (left head-right head). "D" denotes a head with the linker in the post-stroke position and "D*" in the pre-stroke position. Each column contains states with a certain relative position m, n of the two heads' MTBDs (right head is attached m tubulin $\alpha-\beta$ dimers ahead and n protofilaments to the side of the left head). Below each configuration is the corresponding elastic energy.

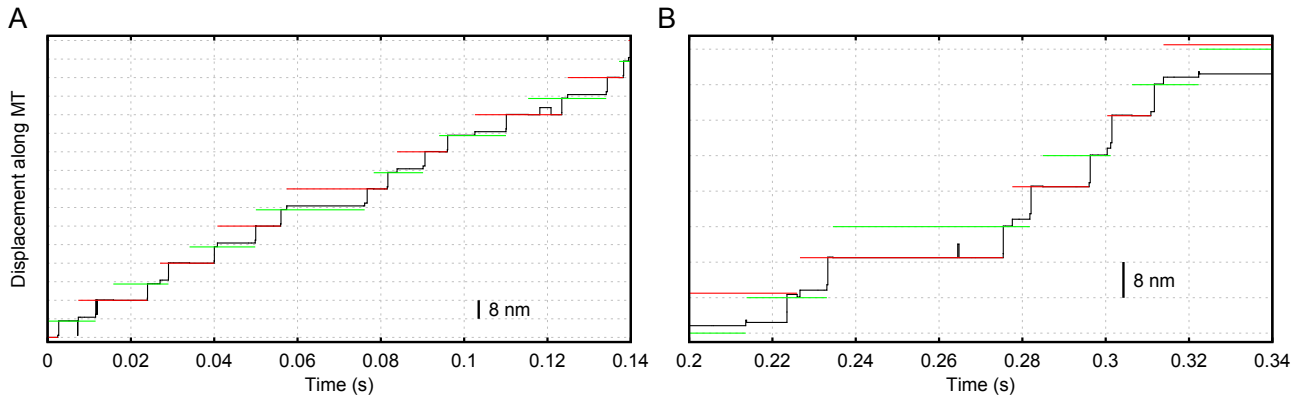


Figure S2: Stepping patterns of tightly coupled dynein dimers. (A) The majority of steps are alternating advancing steps. (B) Occasionally, inch-worm stepping can be observed. x-coordinates of the tail (black), the MTBD of the left (red) and the right (green) head are shown.

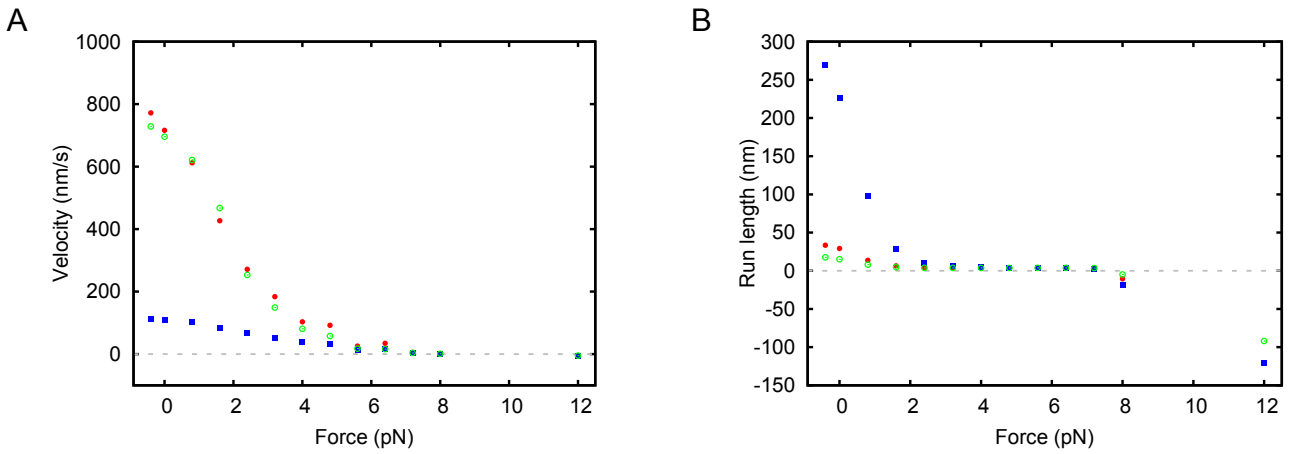


Figure S3: Effect of kinetic parameters on the force velocity relation (A) and run length (B) in the tightly coupled model. The red symbols show data for parameter values from the main text ($k^0_{-\mathrm{ADP}} = 160\,\mathrm{s}^{-1}$, $k^0_{-\mathrm{Pi}} = 5000\,s^{-1}$), green for a reduced phosphate release rate ($k^0_{-\mathrm{Pi}} = 500\,s^{-1}$) and blue for a reduced ADP release rate ($k^0_{-\mathrm{ADP}} = 15\,\mathrm{s}^{-1}$). A slower ADP release rate slows down the overall cycling rate and thus the velocity, but at the same time increases the processivity and the maximum force. A slower Pi release rate increases the dwell time of the lead head in the weakly bound state, which reduces the processivity and maximum force.

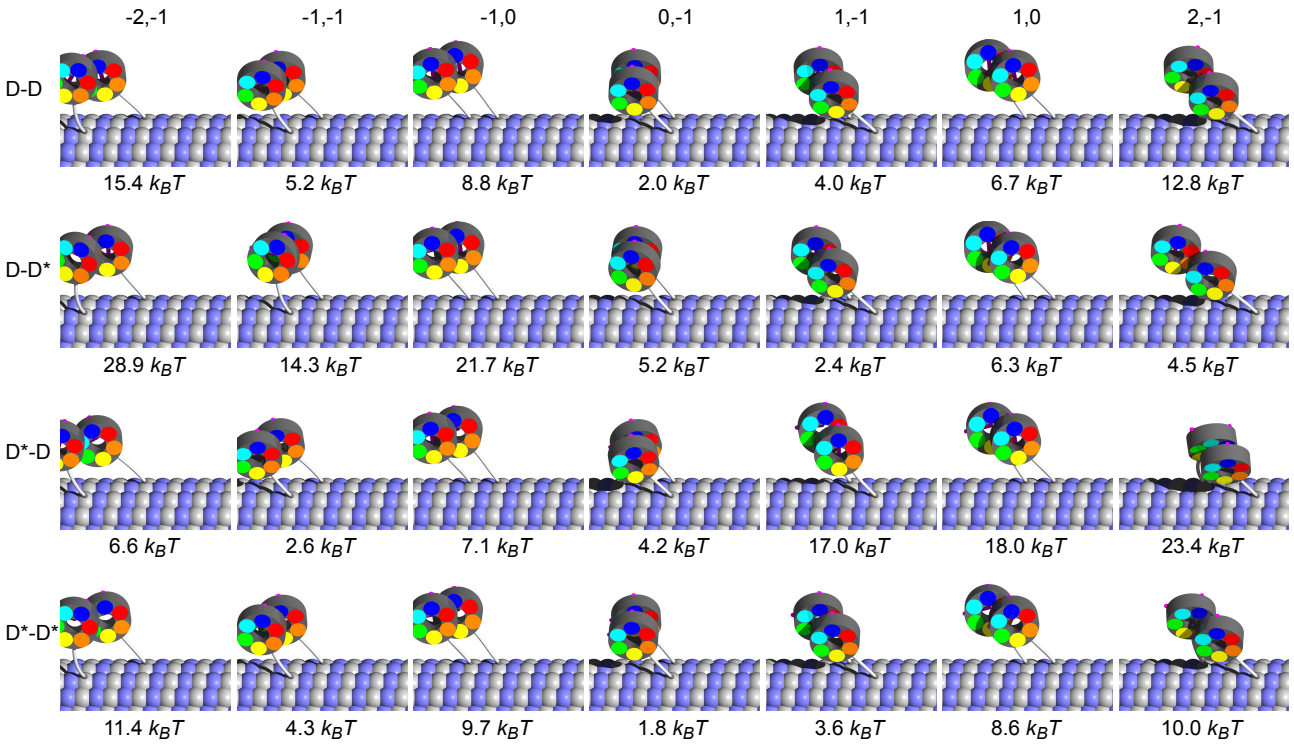


Figure S4: A selection of configurations for the loosly coupled dimeric dynein molecule. See Fig. S1 for a description of labels.

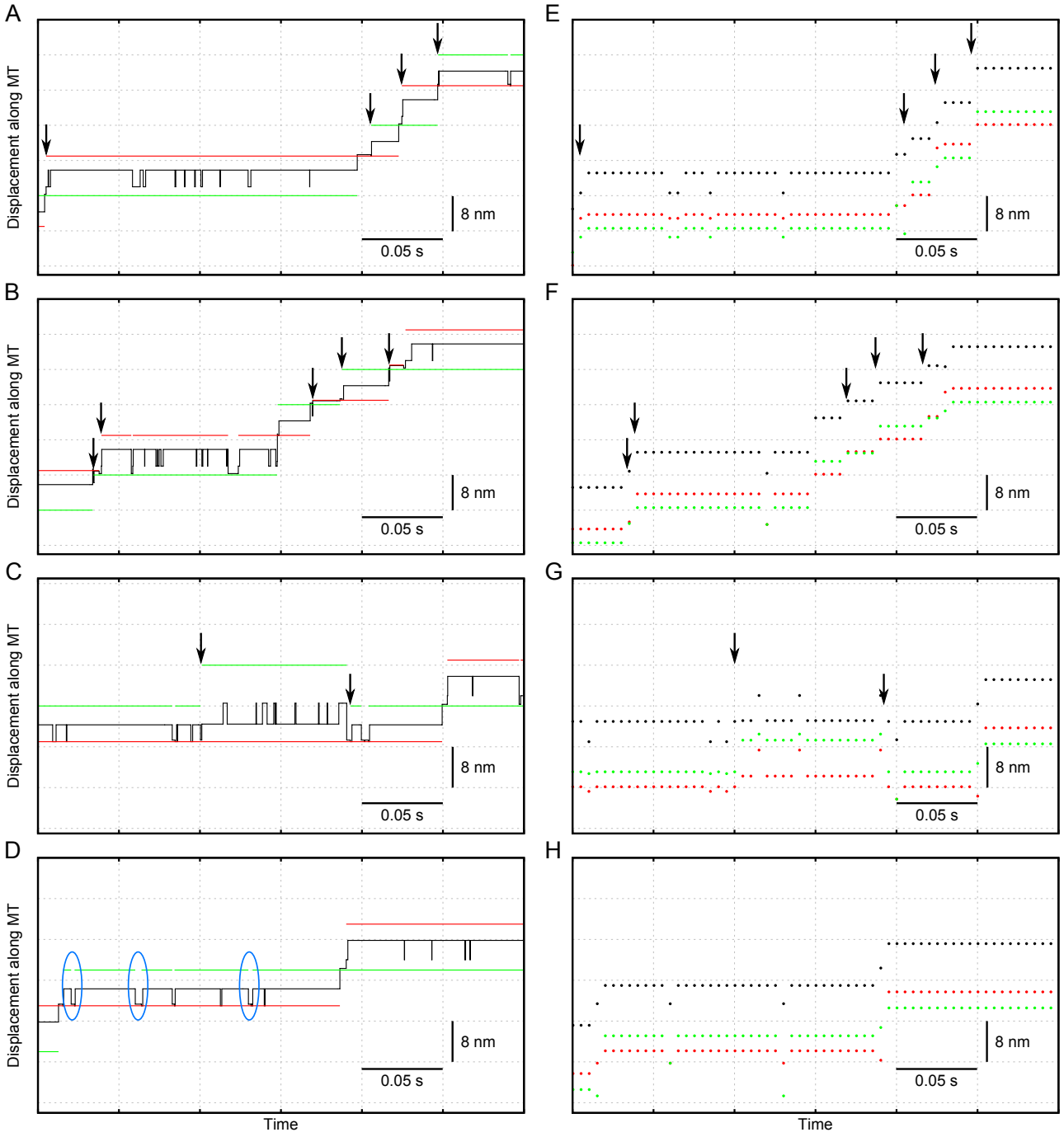


Figure S5: Stepping patterns of loosely coupled dynein dimers (tail: black, left head: red, right head: green). The steps can either be alternating (A,B) or non-alternating (C,D). In (A) the heads pass each other in a hand-over-hand fashion whereas in (B) the inch-worm like behavior can be observed. The non-alternating steps can lead to forward or backward movement (C), or they do not move the motor (D). The futile steps are highlighted by ovals in (D). (E–H) The same as in (A–D) but mimicing the experimentally obtainable information as from signals from quantum dots located at AAA1 domain of left/right head and tail, recorded every 5 ms. The arrows denote representative steps of each kind. One can notice that some of the steps/transitions are masked or even lost which is one of the sources of the noise of the experimental results. The signal from tail is in all plots shifted for 10.5 nm.

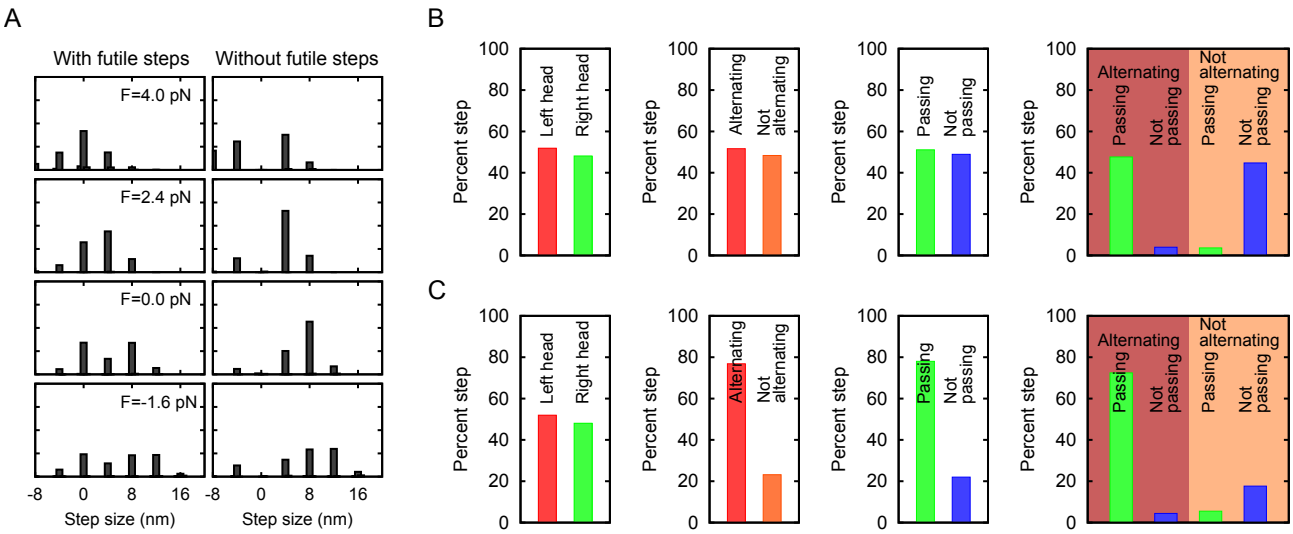


Figure S6: Distributions of steps for loosely coupled dynein dimers. (A) Step size distributions for different loads and taking into account all steps (left panel) or discarding futile (zero length) steps. (B–C) Temporal and spatial analysis of the relative frequency of steps. *Alternating*: current and previous step taken by different heads; *not alternating*: current and previous step taken by the same head; *passing*: the heads switch the lead and trail position; *not passing*: the heads preserve their lead/trail position. (B) Futile steps, which are generally not observable in experiments, are included in the analysis. (C) Futile steps are excluded from the analysis.

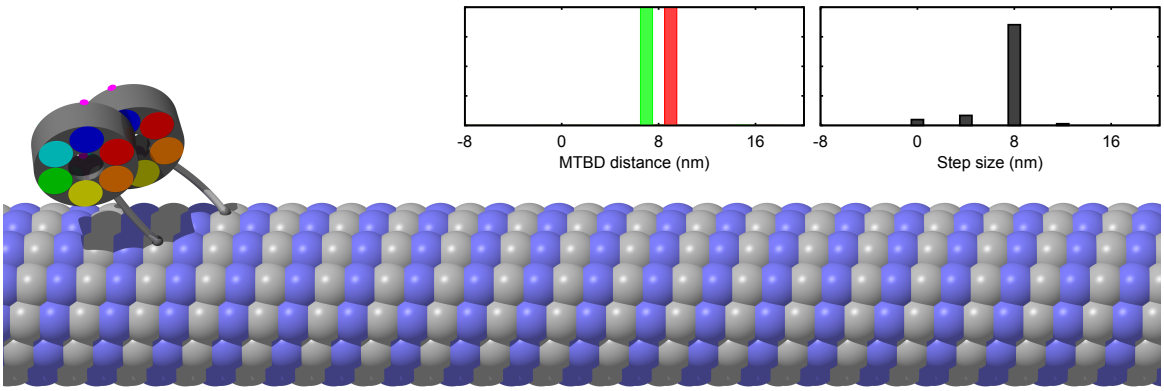
Supporting Tables

Table S1: Parameters for the loosely coupled dimers*

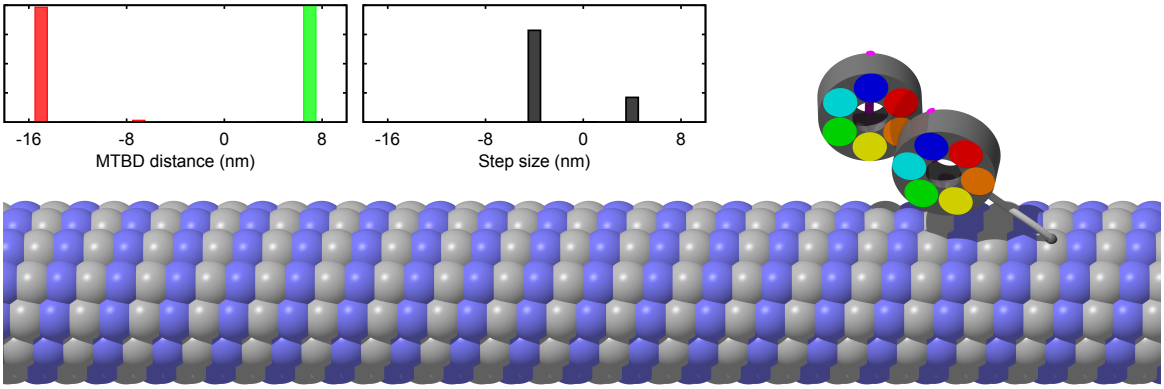
Parameter	Description	Parameter value
k_l	linker-linker interaction	$0.1\,k_BT/\mathrm{nm}^2$
$k^0_{-\mathrm{ADP}}$	ADP release	$15\,\mathrm{s}^{-1}$ †
$k^0_{+\mathrm{ADP}}$	ADP binding	$2.7\times10^5\,\mathrm{M}^{-1}\mathrm{s}^{-1}$

*All other parameters are identical to the tightly coupled model (Tabs. 1, 2, 3).
†Estimated from velocity.

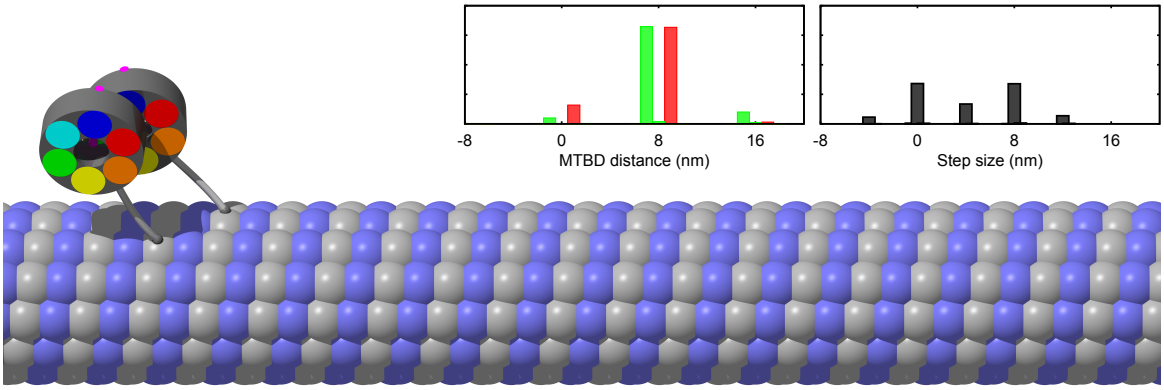
Supporting Movies



Movie S1: Processive motion of an unloaded tightly coupled dynein corresponding to the stepping of mammalian cytoplasmic dyneins; [ATP] = 1 mM. One second of the movie represents 0.01 s real-time.



Movie S2: Processive motion of a tightly bound dynein under superstall load; $F = 12$ pN, [ATP] = 1 mM. One second of the movie represents 0.1 s real time.



Movie S3: Processive motion of an unloaded loosely coupled dynein mimicking the stepping of yeast dyneins; [ATP] = 1 mM. One second of the movie represents 0.1 s real time.