Supporting Information : Mechanical control of the directional stepping dynamics of kinesin motor

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Preparation of kinesin and MT structure : The simulations of kinesin were performed, referenced to an ADP-complexed crystal structure of rat kinesin dimer (PDB code : 3kin) in which the neck-linkers of both monomers are in an ordered state. For the completeness, using a self-avoiding chain, we filled the gap of the missing residues 240-255, whose sequence is sktgaegavld, corresponding to the L12 loop in the crystal structure. Unlike the monomeric kinesin, KIF1A, whose L12 loop contains many lysine residues implying an important role in the motility by interacting with negatively charged E-hook of the tubulin [1], the L12 loop of rat kinesin has only one lysine. Presumably, the electrostatic between the L12 loop and MT surface is not as important as the one in KIF1A. As an initial configuration, one of the kinesin monomers was placed on a tubulin binding site of the MT, and the other monomer is tethered to the MT-bound monomer via coiled-coil association but away from the direct MT interaction range. The topological information of binding interface between kinesin and tubulin was acquired using the Hirokawa and coworkers' KIF1A and tubulin complex [2] (see also Ref. [3] for the detailed procedure).

Computation of two-dimensional potential of mean force (PMF) : The multiple histogram reweighting technique [4, 5] was adopted to compute the two-dimensional PMF between the tethered kinesin head and MT at temperature T. For example, the 2-D PMF can be obtained at arbitrary values of T if the conformational states are well sampled over a range of Tvalues. The probability of finding the kinesin head at position (x, z) at temperature T is given by

$$P(x,z)(T) = \frac{\sum_{E} e^{-E/T} \frac{\sum_{k=1}^{K} h_k(E,x,z)}{\sum_{k=1}^{K} n_k e^{(F_k - E)/T_k}}}{\sum_{E,x,z} e^{-E/T} \frac{\sum_{k=1}^{K} h_k(E,x,z)}{\sum_{k=1}^{K} n_k e^{(F_k - E)/T_k}}}$$
(1)

where K is the number of histograms, $h_k(E, x, z)$ is the number of states between $(E, E + \delta E)$, $(x, x + \delta x)$, and $(z, z + \delta z)$ in the k-th histogram, $n_k = \sum_{E,x,z} h_k(E, x, z)$, T_k is the temperature in the simulations used to generate the k-th histogram. The free energy, F_k , that is calculated self-consistently, satisfies

$$e^{-F_r/T_r} = \sum_{E,x,z} e^{-E/T_r} \frac{\sum_{k=1}^K h_k(E,x,z)}{\sum_{k=1}^K n_k e^{(F_k - E)/T_k}}.$$
(2)

The self-consistent equation for $Z_k \equiv e^{-F_k/T_k}$ converge to the final values of $\{Z_k\}$ starting from

 $Z_k = 1 \ (k = 1, 2, ..., K)$. Using the hybrid MC/MD simulations, we sampled the conformational states over the range of 295 K < T < 356 K. Once P(x, z)(T) is obtained, the 2-D PMF as a function of (x, z) is given by

$$\Delta F(x,y)(T) = -k_B T \log P(x,z)(T).$$
(3)

 $\Delta F(x, y)$, $\Delta F(y, z)$, and $\Delta F(Q_{int}, Q_p)$ are similarly obtained, and the 1-D PMFs are easily reduced from the 2-D PMFs.

Estimate of the translational diffusion constant of kinesin motor domain : When performing the Brownian dynamics simulation, we decided the diffusion constant of monomer using Stokes-Einstein relation,

$$D_o = \frac{k_B T}{6\pi\eta a},\tag{4}$$

where η is the viscosity of water ($\approx 1cP = 10^{-3}N/m^2 \cdot sec$), and a is the hydrodynamic radius of residue ($a \approx 0.19 + 0.14$ nm), so that the monomer diffusion constant at T = 300 K is $D_m \approx 578 \ \mu m^2/sec = 5.8 \times 10^{-6} cm^2/sec$. Similar calculation for the spherical object of radius $R \approx 4$ nm (approximately the size of kinesin head) leads to $D_K \approx 5.5 \times 10^{-7} cm^2/sec = 55$ $\mu m^2/nsec$.

Brownian dynamics with hydrodynamics : To simulate the real time kinetics of kinesin's swiveling motion using a Brownian dynamics of coarse-grained model, the inclusion of hydrodynamics is extremely important to obtain a correct time scale for the translational diffusion of whole object. Without hydrodynamics, the translational diffusion constant of a protein (D_K) that is coarse-grained by the N beads is scaled as D_o/N , where D_o is the diffusion constant of the single bead. To naturally satisfy the Stokes-Einstein relation for the translational diffusion constant, the Brownian dynamics simulations requires the inclusion of hydrodynamic interaction. When off-diagonal elements of diffusional tensor is included and preaveraged, the diffusion constant of whole object D_K scales as [6, 7]

$$D_K = \frac{D_o}{N} + \frac{k_B T}{6\pi\eta R_H} \sim \frac{D_o}{N} + \frac{D_o}{N^\nu} \to \frac{D_o}{N^\nu}.$$
(5)

 $D_K \sim D_o/N^{\nu}$ is the correct scaling relative to the D_o . Without hydrodynamics, the translational diffusion constant is significantly underestimated especially when N is large. However, it is also

challenging to simulate the Brownian dynamics with hydrodynamic interaction. The Langevin equation under multidimensional space is

$$\mathbf{r}_{i}(t+\Delta t) - \mathbf{r}_{i}(t) = \frac{1}{k_{B}T} \sum_{j} \mathbf{D}_{ij} \cdot \mathbf{F}_{j} + \sqrt{2} \sum_{j} \mathbf{B}_{ij} \cdot \mathbf{n}_{j}(t)$$
(6)

with $\langle \mathbf{n}_i(t) \rangle = 0$, $\langle \mathbf{n}_i(t) \cdot \mathbf{n}_i(t) \rangle = \delta_{ij} \delta(t - t')$ and $\mathbf{D} = \mathbf{B}\mathbf{B}^T$. The diffusion tensor \mathbf{D} should be positive definite i.e. $\sum_{ij} \mathbf{F}_i \cdot \mathbf{D}_{ij} \cdot \mathbf{F}_j > 0$ for all $\mathbf{F} \neq 0$, then \mathbf{D} is decomposed into \mathbf{B} and \mathbf{B}^T using the Cholesky decomposition. For the hydrodynamic diffusion tensor, Rotne-Prager diffusion tensor [7] is used. By averaging the square displacement of many different trajectories, we show in Fig.9 how the explicit inclusion of hydrodynamics can alter the translational diffusion constant of the kinesin head domain. The value of $D(hydro) = 19.9 \ \mu m^2/sec$ is closer to the expected value for the $D_K (\approx 55 \ \mu m^2/sec$. See above) that can be roughly estimated from the Stokes-Einstein relation.

Consideration of electrostatics – Manning counterion condensation around the microtubule : The microtubule is viewed as a cylindrical object with many charges. Unlike other thin polyelectrolyte such as RNA, ss-DNA and ds-DNA that can adapt its conformation depending on the salt concentration, the MT can serve as an excellent example to apply the Manning condensation theory for the cylindrical object. Because each tubulin hetero-dimer contains $\sim 35e$ negative charge, the line charge density of microtubule is computed as

$$d \approx \frac{(13 \times 35e)}{80 \text{\AA}} \approx 5.6e/\text{\AA}.$$
(7)

The large Manning condensation parameter [8] $\xi = l_B/b(\approx 7.1 \text{\AA}/(1/5.6) \text{\AA}) \approx 39.8$ suggests that a drastic counterion condensation should occur around the microtubule to make $\xi \approx 1$. This requires $n \sim 34.1e$ monovalent positive counterion, which is estimated from $b^* \approx l_B$, i.e., $\frac{82}{13\times(35-n)} \approx 7.1$, should condense to the surface of tubulin. The numerical solution of nonlinear Poisson-Boltzmann equation with $\phi'(a) = -\frac{2\xi}{a}$ and $\phi(\infty) = 0$

$$\nabla^2 \phi = \kappa^2 \sinh \phi, \tag{8}$$

where $\phi(r) \equiv e\psi(r)/k_BT$ and $\kappa = \sqrt{8\pi l_B c}$, determines the ion distribution around the charged cylinder. With $\phi(r)$ value, the number of positive and negative ions per l_B around the cylinder

can be computed using $n_+(r) = n_{\infty}e^{-\phi}$ and $n_-(r) = n_{\infty}e^{\phi}$, respectively. The net condensate charge number $q(r) = (n_+(r) - n_-(r))$ provides the thickness of condensate ion by imposing the Manning condensation condition, $q(R_M) \times l_B = 1$. For the parameter a = 12.5 nm, c = 150 mM, the counterion condensation occurs at $R_M \approx 16.7 nm$ (see Fig.10). About 4 nm condensate counterion layer is formed around the cylinder. Unless the interfacial binding takes place and expulses the counterion condensated near the binding site, the electrostatic potential due to the microtubule charge does not significantly affect the swiveling dynamics of kinesin head. The widely-accepted notion of "electrostatic steering" in the context of protein-protein association dynamics is not clear for the object on highly charged but effectively neutralized cylindrical surface. It has been shown that the processivity of the kinesin is affected by the salt concentration [9]

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FIG. 7: Mechanochemical cycle of the conventional kinesin at the track of MT protofilament. ATP binding to the leading head (red) induces a undocked-to-docked transition of the neck-linker, which results in 16-nm stepping dynamics of the trailing head (blue). When the tethered head succeeds in binding to the next binding site, the rearward tension built on the neck-linker perturbs the nucleotide binding site and eases the dissociation of ADP from the tethered head [3, 10]. The ATP hydrolysis follows at the trailing head (red). The step corresponding to the stepping motion is enclosed by a box.



FIG. 8: A snapshot of simulation shown in the xz, xy, yz plane. The kinesin monomer in red is the MT-bound head, and the kinesin monomer in blue is the tethered head.



FIG. 9: The diffusion constant of kinesin head domain is computed using Brownian dynamics simulations with explicit hydrodynamic tensor (Rotne-Prager tensor). Mean square displacements ($\langle \delta R^2 \rangle$) of single bead (left) and kinesin head composed of 334 beads (right) are compared. For kinesin head, we performed Brownian dynamics simulations with and without hydrodynamics. By including the hydrodynamics, the correct diffusion behavior of kinesin head is obtained.



FIG. 10: The numerical solution of nonlinear Poisson-Boltzmann equation under varying concentrations of monovalent salt. The electrostatic potential around cylinderical geometry $\phi(r)$ is obtained by solving $\nabla^2 \phi = \kappa^2 \sinh \phi$ with boundary condition, and $\phi(r)$ is used to compute $n_+(r)$, $n_-(r)$, and q(r). The inset and the purple line are the numerical solution for c = 150 mM. The thickness of condense ion layer, R_M , is determined using the Manning condensation criterion, $q(R_M) \times l_B = 1$.