Supporting Information for Landscape-Based View on the Stepping Movement of Myosin VI

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Supporting Text

The simulated system is composed of the leading and rear heads of myosin VI and an actin filament consisting of 26 subunits. We represented each amino acid as a coarse-grained particle (CP). Bound Mg\textsuperscript{2+} and ADP in the prestroke structure M\textsubscript{pre} were represented by all non-hydrogen atoms, whereas M\textsubscript{post} and M\textsubscript{rigor} contained no ligand atoms. The total potential energy was given by

\[ E_{\text{total}}(R_l, R_r, R_a) = E_{\text{intra}}(R_l) + E_{\text{intra}}(R_r) + E_{\text{intra}}(R_a) + E_{\text{inter}}(R_l, R_r) + E_{\text{inter}}(R_l, R_a) + E_{\text{res}}(R_a) + E_{\text{res}}(R_r) + E_{\text{umb}}(R_l), \]  

(S1)

where l, r, and a denote the leading head, the rear head, and the actin filament, respectively, and \( E_{\text{intra}}, E_{\text{inter}}, E_{\text{res}}, \) and \( E_{\text{umb}} \) represent the potential energy functions for the intramolecular and intermolecular interactions, the spatial restraints, and the umbrella potentials, respectively. \( R_l, R_r, \) and \( R_a \) are Cartesian coordinates of CPs in the leading head, the rear head, and the actin filament, respectively.

Intramolecular interactions

The potential energy function for the intramolecular interaction was given by

\[ E_{\text{intra}}(R_x) = E_{\text{ENM}}(R_x) + E_{\text{ligand}}(R_x). \]  

(S2)

Here,

\[ E_{\text{ENM}}(R_x) = \sum_{j=i+1}^{k_1} \frac{1}{2} \left( r_{ij}^x - r_{ij}^0 \right)^2 + \sum_{j=i+2}^{k_2} \frac{1}{2} \left( r_{ij}^x - r_{ij}^0 \right)^2 + \sum_{j=i+3}^{k_3} \frac{1}{2} \Theta \left( r_{ij}^c - r_{ij}^0 \right) \left( r_{ij}^x - r_{ij}^0 \right)^2, \]  

(S3)

is the elastic network model of proteins [1, 2]. x stands for l, r, or a, \( r_{ij} \) denotes distance between CPs within an instantaneous structure, i.e., \( r_{ij}^x = |r_i^x - r_j^x| \), and \( r_{ij}^0 \) denotes the distance in reference structures as defined in the Structure models subsection in the Methods section of the
main text. \( \Theta(x) = 1 \) \((x \geq 0)\) or 0 \((x < 0)\) is the Heaviside step function with \( r^{CP}_{c} = 10 \text{Å}\)\([1, 2]\). Spring constants, \( k_1, k_2, \) and \( k_3, \) were set to the same as in Ref.[2], which are the values estimated to reproduce the B-factors in the X-ray crystallography data and the molecular dynamics simulation data of proteins [1] (Table S1). In cases where the three-helix bundles unfold, we set \( k_3 = 0 \) for the contacts between helices in the three-helix bundle and also for the contacts between the three-helix bundles and the other parts of the two heads of myosin VI by retaining \( k_3 \neq 0 \) for the contacts within each helix (helix 1 comprises the residues 835–862, helix 2 comprises the residues 863–891, and helix 3 comprises the residues 892–909 in each head).

The ligand contact potential for ligands Mg\(^{2+}\) and ADP within the leading head is

\[
E_{\text{ligand}}(R_s) = \sum_{j>i} \frac{k_{\text{ligand}}}{2} \left( r_{ij} - r_{ij}^{(0)} \right)^2,
\]

where the summation goes over both the pair of ligand atoms located within \( r_{c,\text{ligand}} = 4.5 \text{Å} \) from each other in the reference structure and the pair of CP and the ligand atom if any of the atoms in the amino acid residue and the ligand atom are located within \( r_{c,\text{ligand}} = 4.5 \text{Å} \) from each other in the reference structure [3, 4]. The spring constant \( k_{\text{ligand}} \) was set to the same as \( k_1 \). As a whole, \( E_{\text{inter}}(R_s) \) was defined so that the reference structure is the energy-minimum structure of \( E^*_\text{inter}(R_s) \).

### Elastic energies of the SAH domains

The leading and rear heads are connected by two SAH domains, which were implicitly represented as Λ-shaped elastic rods. This assumption enabled us to directly examine the effects of modulating the persistence length of the SAH domain. We approximated intermolecular interactions between the leading head and the rear head by a sum of interactions as

\[
E_{\text{inter}}(R_1, R_r) = E_{\text{distance}} + E_{\text{angle}} + E_{\text{collision}} + E_{\text{torsion}},
\]

We label the position of four residues, 892 of the leading head, 909 of the leading head, 909 of the rear head, and 892 of the rear head, as A, B, C, and D, respectively. Then, we consider the interactions between the two vectors BA and CD. The energy which is dependent only on the distance \( d = \overrightarrow{BC} \) is given by

\[
E_{\text{distance}} = E_{\text{bend}} \Theta(2L - d) + E_{\text{open}} \Theta(2L - d) \Theta \left( d - \frac{4L}{\pi - \theta_0^{\text{center}}} \cos \frac{\theta_0^{\text{center}}}{2} \right) + E_{\text{pull}} \Theta(d - 2L),
\]

where we used Heaviside’s function \( \Theta(x) = 1 \) for \( x \geq 0 \) and \( \Theta(x) = 0 \) for \( x < 0 \). For \( d \leq 2L \), the bending energy of the two elastic rods with the bending angle \( \theta_{\text{bend}} \) is given by

\[
E_{\text{bend}} = 2P k_3 T \theta_{\text{bend}} \left( d \right)^2 \overrightarrow{2L}
\]

where \( P \) is the persistence length of the SAH domain, \( L \) is the length of each elastic rod, and the prefactor of 2 comes from the number of elastic rods in the system. Adopting the value observed in the NMR measurement [5, 6], a rather large value of \( P \) was used as explained in the main text (Table S1). \( \theta_{\text{bend}} = \theta_{\text{bend}}(d) \) can be obtained by numerically solving \( d = d(\theta_{\text{bend}}) \) using the Newton-Raphson method, which requires

\[
d(\theta_{\text{bend}}) = \begin{cases} 
\frac{2L}{2L} \sin \theta_{\text{bend}} & \text{for } 0 \leq d \leq \frac{4L}{\pi - \theta_0^{\text{center}}} \cos \frac{\theta_0^{\text{center}}}{2} \\
\left( \cos \frac{\theta_0^{\text{center}}}{2} - \cos \left( \frac{\theta_0^{\text{center}}}{2} + \theta_{\text{bend}} \right) \right) & \text{for } \frac{4L}{\pi - \theta_0^{\text{center}}} \cos \frac{\theta_0^{\text{center}}}{2} < d \leq 2L.
\end{cases}
\]

For \( 0 \leq d \leq \frac{4L}{\pi - \theta_0^{\text{center}}} \cos \frac{\theta_0^{\text{center}}}{2} \), we assumed that the central angle \( \theta_0^{\text{center}} \) of the Λ-shaped elastic rods is equal to a constant \( \theta_0^{\text{center}} \). For \( \frac{4L}{\pi - \theta_0^{\text{center}}} \cos \frac{\theta_0^{\text{center}}}{2} < d \leq 2L \), we assumed that the central
angle \( \theta_{\text{center}} \) becomes larger than \( \theta_{\text{center}}^0 \) while the other ends (at points B and C) are kept parallel with the straight line BC. This extra opening angle is accompanied by the penalty energy function as \( E_{\text{open}} = k_{\text{open}} (\theta_{\text{center}} - \theta_{\text{center}}^0)^2 \) for \( \theta_{\text{center}} > \theta_{\text{center}}^0 \). For \( d > 2L \), \( \theta_{\text{center}} = 2\pi \) and we consider that two points B and C are pulled toward each other by a spring potential, \( E_{\text{pull}} = k_{\text{pull}} (d - 2L)^2 \).

The angles \( \theta_{ABC} \) and \( \theta_{BCD} \) are subject to the bond angle potential as

\[
E_{\text{angle}} = k_{\text{angle}} (\theta_{ABC} - \theta_{\text{angle}}^0(d))^2 + k_{\text{angle}} (\theta_{BCD} - \theta_{\text{angle}}^0(d))^2
\]

where \( \theta_{ABC} = \cos^{-1} \left( \frac{(\vec{r}_A - \vec{r}_B) \cdot (\vec{r}_C - \vec{r}_B)}{|\vec{r}_A - \vec{r}_B||\vec{r}_C - \vec{r}_B|} \right) \), \( \theta_{BCD} = \cos^{-1} \left( \frac{(\vec{r}_B - \vec{r}_C) \cdot (\vec{r}_D - \vec{r}_C)}{|\vec{r}_B - \vec{r}_C||\vec{r}_D - \vec{r}_C|} \right) \), and

\[
\theta_{\text{angle}}^0(d) = \begin{cases} \frac{\pi + \theta_{\text{angle}}^0}{2} + \theta_{\text{hand}}(d) & \text{for } 0 \leq d \leq \frac{4L}{\pi - \theta_{\text{center}}^0} \cos \frac{\theta_{\text{center}}^0}{2} \\ \frac{\pi}{2} & \text{for } \frac{4L}{\pi - \theta_{\text{center}}^0} \cos \frac{\theta_{\text{center}}^0}{2} < d \end{cases}
\]

It should be noted that \( \frac{\pi + \theta_{\text{angle}}^0}{2} + \theta_{\text{hand}}(d) = \pi + (\pi - \theta_{\text{hand}}(d)) + \theta_{\text{hand}}(d) = \pi \) for \( \frac{4L}{\pi - \theta_{\text{center}}^0} \cos \frac{\theta_{\text{center}}^0}{2} < d \).

We also consider the collision potential as

\[
E_{\text{collision}} = \begin{cases} k_{\text{collision}} (\delta - r_{\text{th}})^2 & \text{for } \delta < r_{\text{th}} \\ 0 & \text{for } \delta \geq r_{\text{th}} \end{cases}
\]

where \( k_{\text{collision}} = 0.75 \text{ kcal/mol/Å}^2 \) and

\[
\delta = \begin{cases} \sqrt{x_B^2 + y_B^2} & \text{for } x_B(x_B - x_C) + y_B(y_B - y_C) \leq 0 \\ \left| \frac{x_B y_C - y_B x_C}{(x_B - x_C)^2 + (y_B - y_C)^2} \right| & \text{for } 0 < x_B(x_B - x_C) + y_B(y_B - y_C) < 1 \\ \sqrt{x_C^2 + y_C^2} & \text{for } 1 \leq x_B(x_B - x_C) + y_B(y_B - y_C) \end{cases}
\]

is the nearest distance of the line segment BC from the z-axis and \( r_{\text{th}} \) is the threshold distance corresponding to the sum of the radii of the actin filament and the SAH domain, which was set to 50 Å.

We further define the torsional potential energy as

\[
E_{\text{torsion}} = \frac{1}{2} k_{\text{torsion}} \left( \frac{\theta_{\text{twist}}}{2L} \right)^2 2L = \frac{k_{\text{torsion}}}{4L} (\theta_{\text{twist}})^2.
\]

The twisting angle \( \theta_{\text{twist}} \) of two SAH domains is defined as follows. We first consider a 180 degrees rotation around an axis with which the vector \( \vec{DC} \) can be compared with the vector \( \vec{AB} \). We assume that this axis passes through the midpoint of the line segment BC and that the direction of the rotational axis \( \mathbf{a} \) is

\[
\mathbf{a} = \frac{\vec{r}_B - \vec{r}_A}{|\vec{r}_B - \vec{r}_A|} + \frac{\vec{r}_C - \vec{r}_D}{|\vec{r}_C - \vec{r}_D|},
\]

where \( \mathbf{a} = |\mathbf{a}| = 2 \cos \frac{\theta_{\text{open}}}{2} \) with \( \theta_{\text{open}} = \theta_{\text{center}} + 2\theta_{\text{hand}} = \cos^{-1} \left( \frac{(\vec{r}_B - \vec{r}_A) \cdot (\vec{r}_C - \vec{r}_D)}{|\vec{r}_B - \vec{r}_A||\vec{r}_C - \vec{r}_D|} \right) \). We further define two residues P and Q, which are residue 837 in the leading head and the rear head, respectively. We consider the six points, A, B, and P in the leading head and C', D', and Q', which are the rotated image of C, D, and Q in the rear head. When we view the six points from B toward A (also from C' toward D'), the angle PBQ' = the angle PC'Q' is projected onto a plane vertical to the vector B\( \vec{A} \) (and also to C'D'). This angle is defined to be the twisting angle, the sign of which is defined to be positive for the clockwise rotation of C'D' from B\( \vec{A} \). We define \( \theta_{\text{twist}} \) as

\[
\theta_{\text{twist}} = \left( \frac{\rho_{PB}^P \times \rho_{QC}^C}{|\rho_{PB}^P \times \rho_{QC}^C|} \right) \cdot (\vec{r}_A - \vec{r}_B) \cos^{-1} \left( \frac{\rho_{PB}^P \cdot \rho_{QC}^C}{|\rho_{PB}^P| |\rho_{QC}^C|} \right),
\]

\( \text{(S15)} \)
where $\mathbf{p}_{AB} = (r_p - r_B) \times (r_A - r_B)$ and $\mathbf{p}_{AB}^{Q'} = (r_Q - r_C) \times (r_A - r_B)$. With $\frac{(\mathbf{p}_{AB} \times \mathbf{p}_{AB}^{Q'}) \cdot (r_A - r_B)}{|(\mathbf{p}_{AB} \times \mathbf{p}_{AB}^{Q'}) \cdot (r_A - r_B)|}$ being $1$ or $-1$, we have $-\pi \leq \theta_{twist} \leq \pi$. The positive/negative sign of $\theta_{twist}$ corresponds to the clockwise/counterclockwise rotation of $r_Q - r_C$ with respective to $r_p - r_B$ when these two vectors are viewed from B to A. Modest values were chosen for the parameters, $k_{\text{open}}, k_{\text{pull}}, k_{\text{angle}},$ and $k_{\text{torsion}}$, allowing flexible deformation of the SAH domains with the energy penalty of a few $k_B T$ for a considerable deformation, but a large value of $k_{\text{collision}}$ was used to avoid the collision between the SAH domains and the actin filament (Table S1).

**Intermolecular interactions**

The potential energy for the intermolecular interaction between the leading head and the actin filament is composed of the Debye-Hückel electrostatic interactions and van der Waals interactions as

$$E_{\text{inter}}(\mathbf{R}_i, \mathbf{R}_j) = \sum_{i,j} \frac{k_{\text{ele}} q_i q_j}{r_{ij}} \exp \left( -\frac{r_{ij}}{\rho_l} \right)$$

$$\quad + \sum_{i,j} k_{\text{vdw}} \left[ \left( \frac{r_{\text{vdw}}}{r_{ij}} \right)^{12} - 2 \left( \frac{r_{\text{vdw}}}{r_{ij}} \right)^6 \right],$$

where $r_{ij} = |r_i - r_j|$. $q_i$ is the charge of CP ($-1$ for Asp and Glu, $+1$ for Lys and Arg, and $+0.5$ for His) or the charge of ligand atoms ($-1$ for each of the three oxygen atoms in ADP and $+2$ for Mg$^{2+}$). The cutoff parameters for the calculation were set to much larger than $l_d$ and $r_{\text{vdw}}$ as $r_{\text{ele}} = 160 \text{ Å}$ and $r_{\text{vdw}} = 15 \text{ Å}$.

**Restraint potentials**

The restraint potentials applied to the actin filament and the motor domain of the rear head are given by

$$E_{\text{ra}}(\mathbf{R}_a) = \sum_{i,j} k_{\text{ra}} \left[ (x_{a,i} - x_{a,i}^0)^2 + (y_{a,i} - y_{a,i}^0)^2 + (z_{a,i} - z_{a,i}^0)^2 \right],$$

$$E_{\text{rr}}(\mathbf{R}_r) = \sum_{i,j} k_{\text{rr}} \left[ (x_{r,i} - x_{r,i}^0)^2 + (y_{r,i} - y_{r,i}^0)^2 + (z_{r,i} - z_{r,i}^0)^2 \right],$$

where $(x_{a,i}^0, y_{a,i}^0, z_{a,i}^0)$ and $(x_{r,i}^0, y_{r,i}^0, z_{r,i}^0)$ are the coordinates of the reference structure (see the Model structures subsection of the Methods section in the main text). The similar value of $k_{\text{ra}}$ to the one used in [2] was used and the value of $k_{\text{ra}}$ was set to make the restraint soft enough (Table S1).

**Potentials for umbrella sampling**

We defined the cylindrical coordinate system around the actin filament, $(\theta, z, r)$, where $\theta$, $z$, and $r$ denote the azimuth, the axial coordinate, and the radial distance of the mass center of the motor domain of the leading head with respect to the central axis of the actin filament. To effectively sample the configurational space around actin filament, we applied the umbrella potential given by

$$E_{\text{umb}}(\mathbf{R}_r) = \frac{k_{\text{umb}}}{2} (\theta - \theta_i)^2 + \frac{k_{\text{umb}}}{2} (z - z_j)^2 + \frac{k_{\text{umb}}}{2} (r - r_k)^2,$$

with $\theta_i = \theta_{\text{rear head}} - \frac{7\pi}{8} + \frac{\pi}{4} i (i = 0, \cdots, 7)$, $z_j = -517 + 80 j \text{ Å} (j = 0, \cdots, 6)$, and $r_k = 60 + 15k \text{ Å} (k = 0, 1, 2)$. 

S4
Parameters

Values of the parameters used in the potential energy functions are summarized in Table S1.

| Parameters                      | Values                                      | Remarks                                      |
|--------------------------------|---------------------------------------------|----------------------------------------------|
| Intramolecular interactions    |                                             |                                              |
| 1st neighbor                   | \( k_1 = 6 \times 10^3 \text{pN/nm} \)     | Refs.\([1, 2]\)                              |
| 2nd neighbor                   | \( k_2 = 3 \times 10^3 \text{pN/nm} \)     | Refs.\([1, 2]\)                              |
| 3rd neighbor                   | \( k_3 = 6 \times 10^2 \text{pN/nm} \)     | Refs.\([1, 2]\)                              |
| cutoff distance                | \( r_{CP} = 10 \text{Å} \)                | Refs.\([1, 2]\)                              |
| ligand-ligand & ligand-protein | \( k_{\text{ligand}} = 6 \times 10^3 \text{pN/nm} \) | The same value as \( k_1 \)                   |
| cutoff distance                | \( r_{\text{ligand}} = 4.5 \text{Å} \)    | Refs.\([3, 4]\)                              |
| Elastic energies of the SAH domains |                                             |                                              |
| Elastic constant for varying the center angle | \( k_{\text{open}} = 0.18 \text{kcal/mol/rad}^2 \) | \( 3k_B T \) energy rise for \( \pi \) deviation from the reference angle |
| Elastic constant for rod stretching | \( k_{\text{pull}} = 0.0011 \text{kcal/mol/Å}^2 \) | \( 3k_B T \) energy rise for 4 nm deviation from \( 2L \) |
| Elastic constant for varying the tip angle | \( k_{\text{angle}} = 6.7 \text{kcal/mol/rad}^2 \) | \( 3k_B T \) energy rise for \( \pi/6 \) deviation from the reference angle |
| Elastic constant for torsion    | \( k_{\text{torsion}} = 98.5L \text{kcal/mol/rad}^2 \) | \( 3k_B T \) energy rise for \( \pi/9 \) twisting |
| Repulsion between the rod and the actin filament | \( k_{\text{collision}} = 0.75 \text{kcal/mol/Å}^2 \) | \( 5k_B T \) energy rise in collision within 2 Å |
| Persistence length of the SAH rod | \( P = 22.4 \text{nm} \) | NMR data, Refs.\([5, 6]\)                     |
| Intermolecular interactions    |                                             |                                              |
| Electrostatic interaction strength | \( k_{\text{ele}} = 4.48 \text{kcal/Å/mol} \) | From the relative permittivity \( \varepsilon = 74 \) in water at 310 K |
| Debye length                   | \( l_D = 19 \text{Å} \)                    | 25 mM KCl solution at 310 K                  |
| van der Waals interaction strength | \( k_{\text{vdw}} = 0.0133 \text{kcal/mol Å} \) | Refs.\([3, 4]\)                              |
| van der Waals radius           | \( r_{\text{vdw}} = 8 \text{Å} \)         | Refs.\([3, 4]\)                              |
| Restraint potentials           |                                             |                                              |
| Restraint of the actin filament | \( k_{a, \text{res}} = 1.6 \text{pN/nm} \) | Restraint is soft enough but preventing the filament from moving away thermally. |
| Restraint of the rear head     | \( k_{r, \text{res}} = 1.6 \times 10 \text{pN/nm} \) | Ref.\([2]\)                                  |
| Potentials for umbrella sampling |                                             |                                              |
| Umbrella for \( \theta \)     | \( k_{\text{umb1}} = 0.9877 \text{kcal/mol/rad}^2 \) | Allowing the localized but overlapped sampling over multiple blocks |
| Umbrella for \( z \)          | \( k_{\text{umb2}} = 8.33 \times 10^{-5} \text{kcal/mol/Å}^2 \) |                                          |
| Umbrella for \( r \)          | \( k_{\text{umb3}} = 1.48 \times 10^{-2} \text{kcal/mol/Å}^2 \) |                                          |

Simulations

With each umbrella potential, the Langevin molecular dynamics calculation was performed for 1,000,000 steps with mass \( m = 1.0 \), the friction coefficient \( \gamma = 0.005 \), temperature \( T = 300\text{K} \), and the step size of \( \Delta t = 0.0175 \). The integration scheme was that of Honeycutt and Thirumalai \([7]\). Data for the initial 500,000 steps were discarded as the equilibration process. The remaining data were combined by the weighted histogram analysis method \([8]\) to obtain the free energy landscapes.
References

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Supporting Figures

Figure S1: The free energy landscape obtained for the Houdusse-Sweeney model. The leading head is in the poststroke state.

Figure S2: The free energy landscape obtained for the Houdusse-Sweeney model with the elastic constant of the twisting deformation of the SAH, $k_{\text{torsion}} = 197 L \text{kcal/mol/rad}^2$, which is twice the standard value in Table S1. The leading head is in the prestroke state.
Figure S3: The free energy landscape obtained for the Spudich model except that the three-helix bundles are unfolded. The leading head is in the poststroke state.

Figure S4: The free energy landscape obtained for the Houdusse-Sweeney model except that the three-helix bundles are folded. The leading head is in the poststroke state.
Figure S5: The free energy landscape for the Spudich model with the persistence length modified from 22.4 nm to 5 nm. The leading head is in the poststroke state.