Supporting Text

1. An example of high-dimensional kinetic networks

The kinetic scheme for a double-headed myosin can be complex. Each head can go through kinetic transitions as shown in Scheme 6 of the main text. A state for a double-headed myosin should include independent states in both heads. For example, if the head 1 is in a state with ATP bound but away from actin, and the head 2 is in a state with ADP bound and actin bound, the state can be written as MT-AMD. Since each head has 8 possible states in Scheme 6, the double-headed myosin has $8^2 = 64$ states. Of course some states in this kinetic network are not populated due to the mechanical constraints between two heads. Each state has 6 possible transition directions as shown in Figure 5, namely forward and backward ATP hydrolysis cycle in each head and the change of actin affinity in each head. The whole kinetic network for the double-headed myosin is illustrated in Figure 6.

Though the kinetic network is complex, the procedure of the EAB method to compute the dwell-time distributions is the same. The only task is to assign or hypothesize where the power stroke steps are in the network, and then introduce the absorbing boundary states accordingly to calculate the population of states exiting a dwell.

2. Analytical solution of dwell-time distributions for the three-state kinetic scheme

The reaction for the first-passage time of a cyclic kinetic scheme with three states is

$$\mathrm{B'} \xleftarrow{k_{CB}} \mathrm{C} \underset{k_{AC}}{\overset{k_{CA}}{\rightleftharpoons}} \mathrm{A} \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} \mathrm{B} \xrightarrow{k_{BA}} \mathrm{C'} \tag{1}$$

The equations are

$$\begin{aligned}
\frac{d[\mathrm{B'}]}{dt} &= k_{CB}[\mathrm{C}] \\
\frac{d[\mathrm{C}]}{dt} &= -k_{CA}[\mathrm{C}] + k_{AC}[\mathrm{A}] - k_{CB}[\mathrm{C}] \\
\frac{d[\mathrm{A}]}{dt} &= -k_{AB}[\mathrm{A}] + k_{BA}[\mathrm{B}] - k_{AC}[\mathrm{A}] + k_{CA}[\mathrm{C}] \\
\frac{d[\mathrm{B}]}{dt} &= -k_{BC}[\mathrm{B}] - k_{BA}[\mathrm{B}] + k_{AB}[\mathrm{A}] \\
\frac{d[\mathrm{C'}]}{dt} &= k_{BC}[\mathrm{B}]
\end{aligned} \tag{2}$$

The three equations for [A], [B] and [C] should be solved together so that we can calculate the dwell-time distributions from $\frac{d[\mathrm{B'}]}{dt}$ and $\frac{d[\mathrm{C'}]}{dt}$. Ether by the Laplace transform method or by the eigenvalue method, the analytical solutions for [A], [B] and [C] can be written as a summation of exponentials as:

$$\begin{bmatrix} A \\ B \\ C \end{bmatrix} = c_1 \begin{pmatrix} \alpha_1 \\ \beta_1 \\ \gamma_1 \end{pmatrix} e^{\lambda_1 t} + c_2 \begin{pmatrix} \alpha_2 \\ \beta_2 \\ \gamma_2 \end{pmatrix} e^{\lambda_2 t} + c_3 \begin{pmatrix} \alpha_3 \\ \beta_3 \\ \gamma_3 \end{pmatrix} e^{\lambda_3 t} \tag{3}$$

where λ_1, λ_2 and λ_3 are eigenvalues and $\begin{pmatrix} \alpha_i \\ \beta_i \\ \gamma_i \end{pmatrix}$ are corresponding eigenvectors. The coefficients c_1, c_2 and c_3 are determined by the initial conditions. The eigenvalues λ_1, λ_2 and λ_3 are solutions of a cubic polynomial:

$$\begin{aligned} \lambda_1 &= \sqrt[3]{\alpha} - e^{\frac{2\pi i}{3}} \sqrt[3]{\beta} \\ \lambda_2 &= e^{\frac{2\pi i}{3}} \sqrt[3]{\alpha} - \sqrt[3]{\beta} \\ \lambda_3 &= e^{\frac{4\pi i}{3}} \sqrt[3]{\alpha} - e^{\frac{4\pi i}{3}} \sqrt[3]{\beta} \end{aligned} \tag{4}$$

where

$$\begin{aligned} \alpha &= \frac{-q + \sqrt{q^2 + 4p^3}}{2} \\ \beta &= \frac{q + \sqrt{q^2 + 4p^3}}{2} \end{aligned} \tag{5}$$

where

$$\begin{aligned} p &= \frac{3c - b^2}{9} \\ q &= \frac{2b^3 - 9bc + 27d}{27} \end{aligned} \tag{6}$$

where

$$\begin{aligned} b &= k_{AB} + k_{BA} + k_{BC} + k_{CB} + k_{CA} + k_{AC} \\ c &= k_{CA}k_{AB} + k_{CB}k_{AB} + k_{CB}k_{AC} + k_{AB}k_{BC} + k_{AC}k_{BC} + k_{AC}k_{BA} + \\ &\quad k_{CA}k_{BC} + k_{CA}k_{BA} + k_{CB}k_{BC} + k_{CB}k_{BA} \\ d &= k_{CA}k_{AB}k_{BC} + k_{CB}k_{AB}k_{BC} + k_{CB}k_{AC}k_{BC} + k_{CB}k_{AC}k_{BA} \end{aligned} \tag{7}$$

With the eigenvalues solved, the eigenvectors can thus be obtained by solving the algebraic equations.

3. Parameters for the global fitting of dwell-time distributions for single-headed myosin V

The kinetic scheme for a single-headed myosin shown in Scheme 6 has 8 states. Using this scheme is, however, not successful in globally fitting to dwell-time distributions subject to 6 different experimental conditions (1). We had to add two more states in the scheme in order to fit all data at once:

$$\begin{array}{ccccccccccccc} \ldots & \leftrightarrow & \mathrm{M} & \leftrightarrow & \mathrm{MT} & \leftrightarrow & \mathrm{MDP} & \leftrightarrow & \mathrm{MD^*} & \leftrightarrow & \mathrm{MD} & \leftrightarrow & \mathrm{M} & \leftrightarrow \ldots \\ & & \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow & \\ \ldots & \leftrightarrow & \mathrm{AM} & \leftrightarrow & \mathrm{AMT} & \leftrightarrow & \mathrm{AMDP} & \leftrightarrow & \mathrm{AMD^*} & \leftrightarrow & \mathrm{AMD} & \leftrightarrow & \mathrm{AM} & \leftrightarrow \ldots \end{array} \tag{8}$$

The justification and the detailed explanations for the state addition will be shown in a coming publication. The power stroke is assumed to be in the transition AMD*→AMD, while the swinging back to the pre-stroke conformation is assumed to be in the transition

M→MT. The rates found for global fitting are listed in Table 1. The unit for these rates without involving ligand concentrations is s^{-1}, and the unit for those involving ligand concentrations (e.g., M→MT) is $M^{-1}s^{-1}$. All rates were chosen to satisfy detailed balance in any closed cycle and some rates were obtained from literature (2, 3). To model the effects of forces exerted by the optical trap, we used Boltzmann factors $e^{\alpha F\Delta x/k_BT}$ and $e^{(\alpha-1)F\Delta x/k_BT}$ for a proposed power stroke step to account for the energy surface adjustments due to forces, where Δx is the effective distance in the projected force direction, α is the proportion of the position of the transition state. The value used for Δx is 18 nm, half of the power stroke distance, and the value used for α is 0.5, assuming the transition state is in the middle of the energy surface.

References

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