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Supplementary Materials for

A hidden state in the turnover of a functioning membrane protein complex

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Supplementary Materials

Section S1. The dwell time distribution for the two-state model

We followed a procedure described previously (18). Assume that the motor has *N* stators bound at *t*=0, and define $Q_N(\tau)$ as the subsequent probability that the motor still has *N* stators at *t*=τ>0. Then

$$\frac{dQ_N(\tau)}{d\tau} = -k_1(M-N)Q_N(\tau) - k_{-1}NQ_N(\tau)$$

where *M* is the maximum binding sites per motor. Thus

$$Q_N(\tau) = Ae^{-[k_1(M-N)+k_{-1}N]\tau}$$

where *A* is a normalization constant. From $Q_N(\tau)$, the dwell time distribution $P_N(\tau)$ can be obtained as

$$P_N(\tau) = -\frac{dQ_N(\tau)}{d\tau} = Ce^{-[k_1(M-N)+k_{-1}N]\tau}$$

where *C* is a normalization constant. Therefore $P_N(\tau)$ is a single-exponential function.

Section S2. The dwell time distribution for the three-state model

Assume that the motor has *N* stators bound (in the "O" state) at *t*=0, and define $Q_N(\tau)$ as the subsequent probability that the motor still has *N* stators at *t*=τ>0. Then

$$\frac{dQ_N(\tau)}{d\tau} = -k_1(M-N)Q_N(\tau) - (k_{-1}+k_{-2})NQ_N(\tau) - k_2hQ_N(\tau)$$

where *M* is the maximum binding sites per motor, and *h* is the number of stators in the hidden state "H" per motor. Thus

$$Q_N(\tau) = Ae^{-[k_1(M-N)+(k_{-1}+k_{-2})N+k_2 h]\tau}$$

where *A* is a normalization constant. From $Q_N(\tau)$, the dwell time distribution $P_N(\tau)$ can be obtained as

$$P_N(\tau) = -\sum_h \frac{dQ_N(\tau)}{d\tau}$$

The summation is from $h = 0$ to h_M, the maximum number of stators in the hidden state. Thus $P_N(\tau)$ is a sum of (h_M +1) exponentials. As the shape of the dwell time distribution measured experimentally is a sum of two exponentials, h_M seems to be 1. This suggested that the rate constant k_2 is much larger than the other rate constants such that the number of stators in the hidden state is at most one. Therefore

$$\begin{aligned} P_N(\tau) &= -\sum_{h=0}^{1} \frac{dQ_N(\tau)}{d\tau} \\ &= C_1 e^{-[k_1(M-N)+(k_{-1}+k_{-2})N]\tau} + C_2 e^{-[k_1(M-N)+(k_{-1}+k_{-2})N+k_2]\tau} \\ &\approx C_1 e^{-[k_1(M-N)+(k_{-1}+k_{-2})N]\tau} + C_2 e^{-k_2\tau} \end{aligned}$$

where C_1 and C_2 are two normalization constants.

Supplemental figures

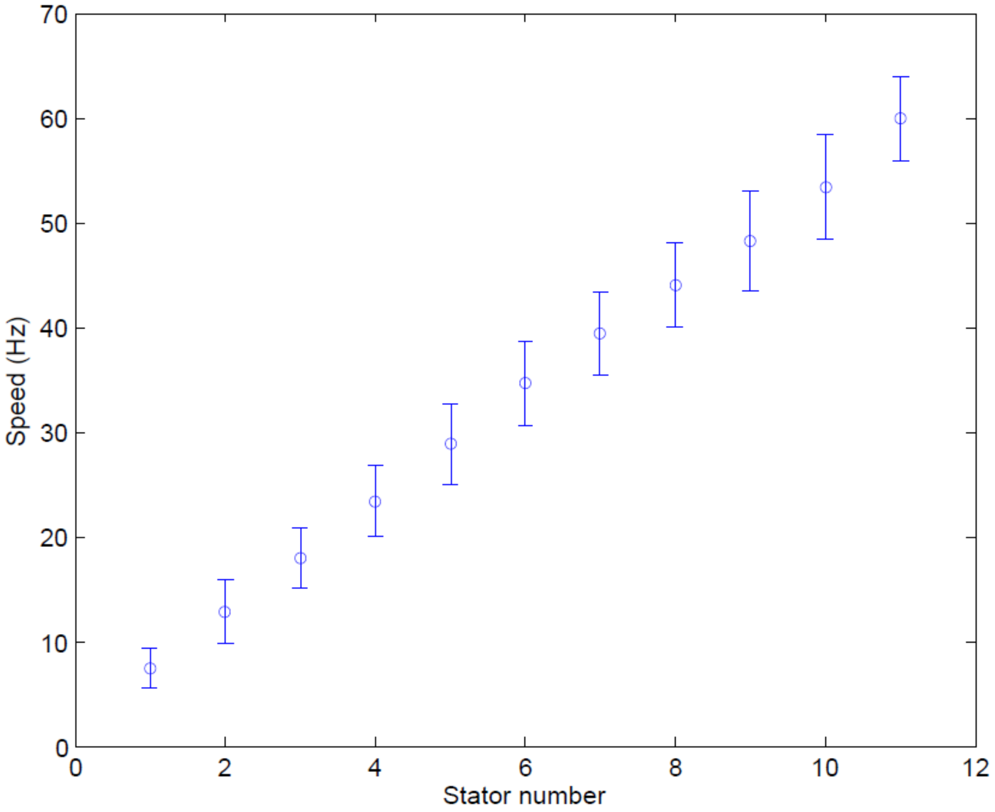

Fig. S1. The motor speed at high load as a function of the number of stators.

Error bars are the standard deviation.

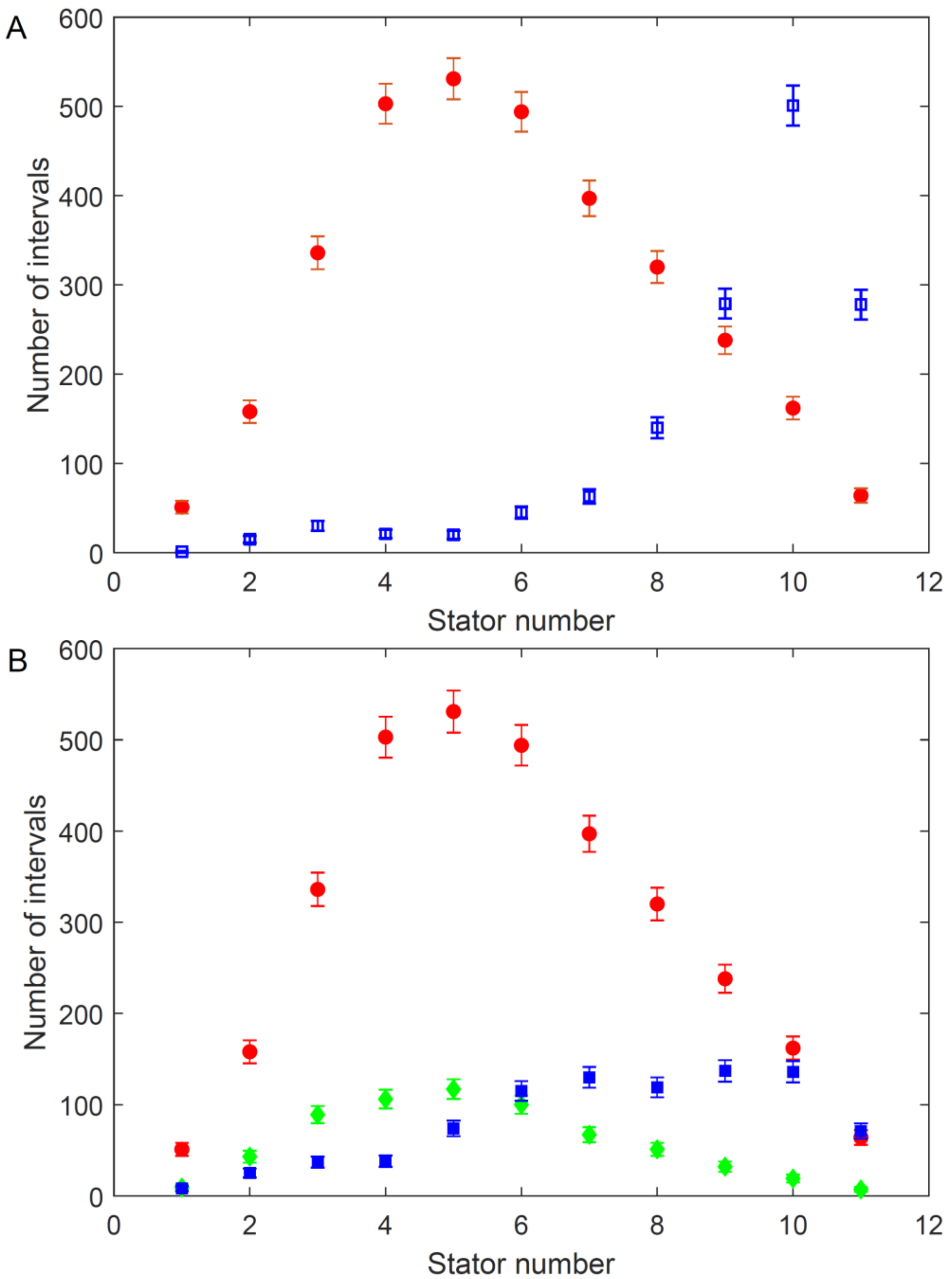

Fig. S2. The distributions of stator number for motors at steady states at various induction levels. (**A**) The distribution of stator number for motors at steady states measured for the mutant strain JY21 induced with 2 μM IPTG (red solid circles) and the wild-type strain JY27 (blue squares). (**B**) The distribution of stator number for motors at steady states measured for the mutant strain JY21 induced with 0, 2, and 5 μM IPTG (green diamonds, red solid circles, and blue filled squares, respectively). The error bars are standard deviations.

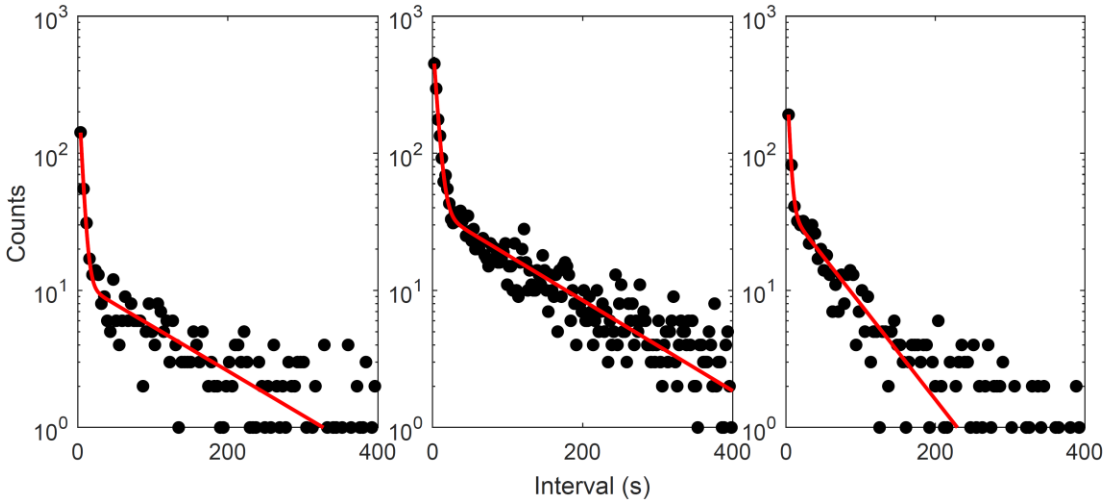

Fig. S3. The dwell time distribution at stator numbers from 1 to 11 for JY21 induced with 0, 2, and 5 μM IPTG (from left to right). The red line is the fit with Eq.1 in the main text.

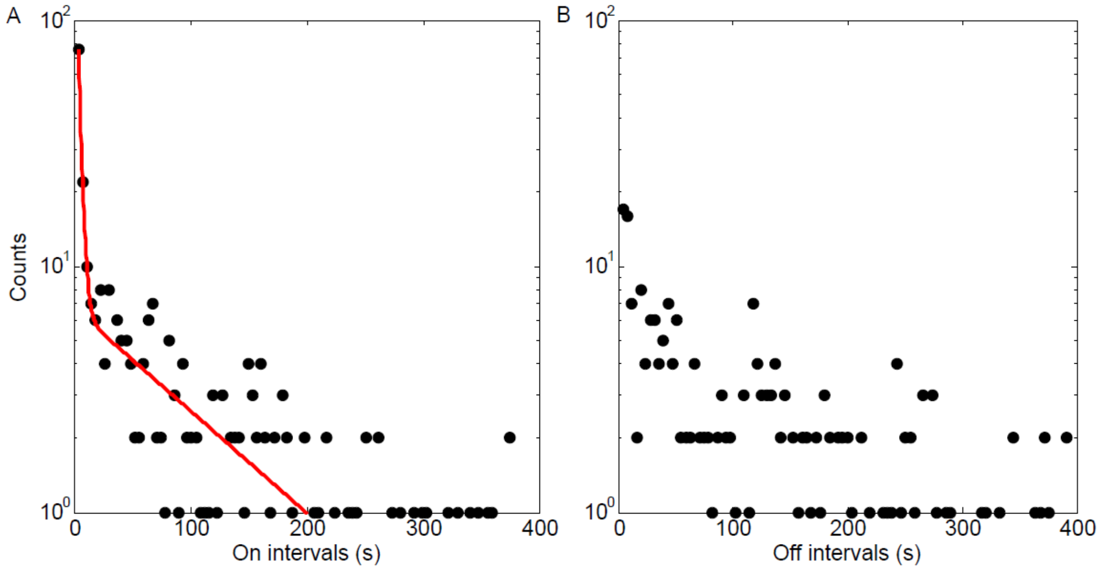

Fig. S4. The dwell time distributions for two types of intervals. The dwell time distributions at stator number of five for “On” intervals (**A**), which are defined as intervals that are followed by a speed increase, and “Off” intervals (**B**), which are defined as intervals that are followed by a speed decease. The red line is a fit with a sum of two exponentials.

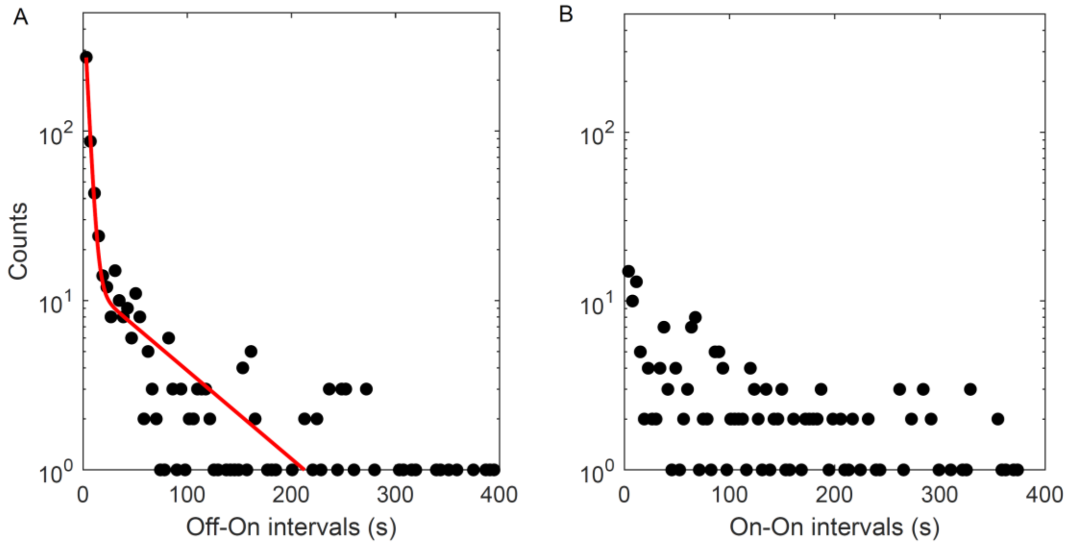

Fig. S5. The dwell time distributions for the two types of "On" intervals. The dwell time distributions at stator number of 4, 5, and 6 for "Off-On" intervals (**A**), which are defined as the "On" intervals that are preceded by a speed decrease, and "On-On" intervals (**B**), which are defined as the "On" intervals that are preceded by a speed increase. The red line is a fit with a sum of two exponentials.

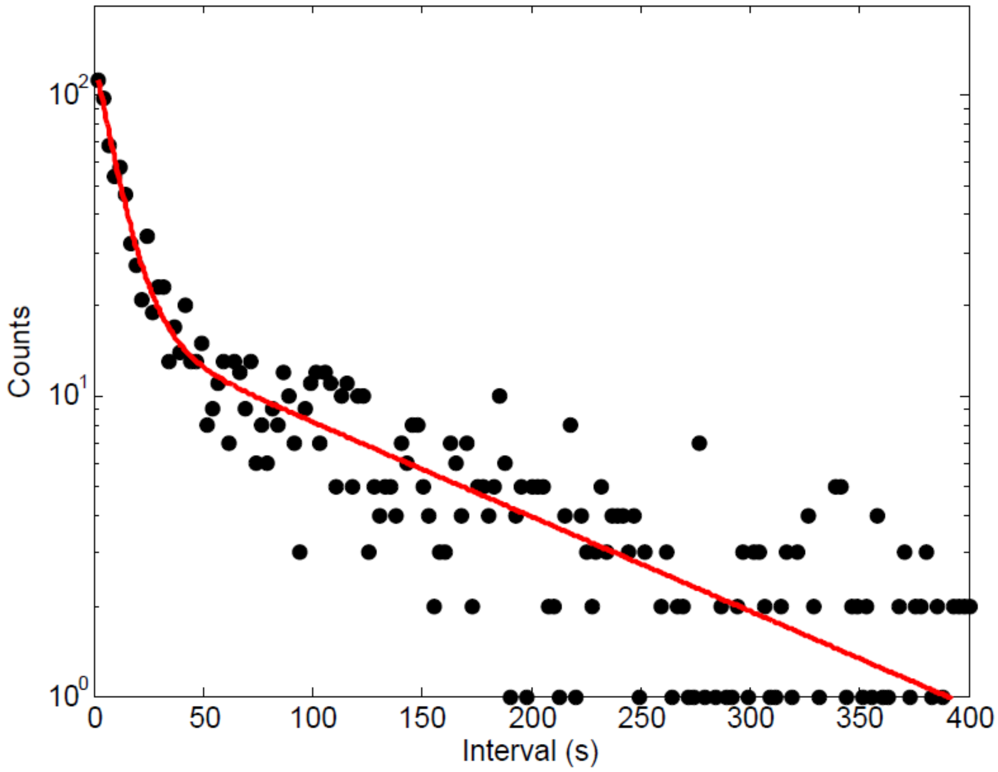

Fig. S6. The dwell time distribution at stator numbers from 1 to 11 for the wild-type strain JY27. The red line is the fit with Eq.1 in the main text, with the results of fitting k_s = 0.0076 ± 0.0006 s^{-1} and k_f = 0.15 ± 0.04 s^{-1}.

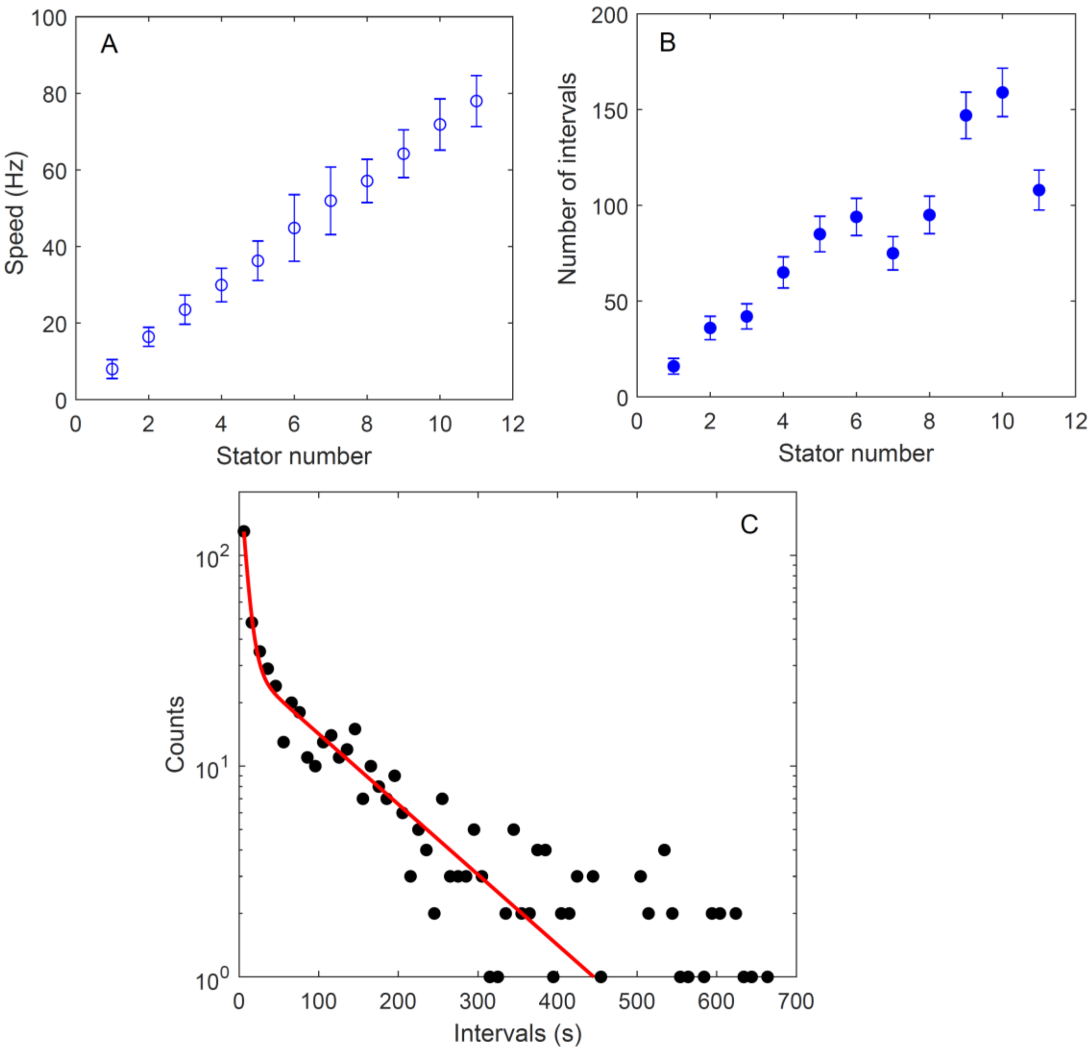

Fig. S7. Data from the MotB plug-deletion strain (SM1 carrying pBAD33MotBΔplug) with induction of 0.001% arabinose. (**A**). The motor speed at high load as a function of the number of stators. Error bars are the standard deviation. (**B**). The distribution of stator number for motors at steady states. (**C**). The dwell time distribution at stator numbers from 1 to 11. The red line is the fit with Eq.1 in the main text, with the results of fitting $k_s = 0.0077 \pm 0.0006$ s^{-1} and $k_f = 0.15 \pm 0.05$ s^{-1}.

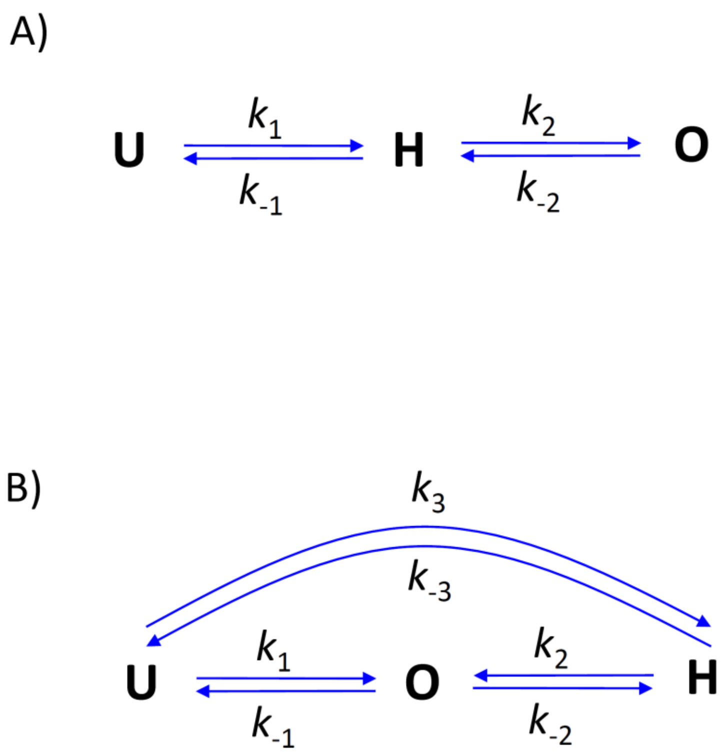

Fig. S8. Other possible three-state models. (**A**) The hidden state “H” is an intermediate between the states “U” and “O”. (**B**) The most general three-state model.

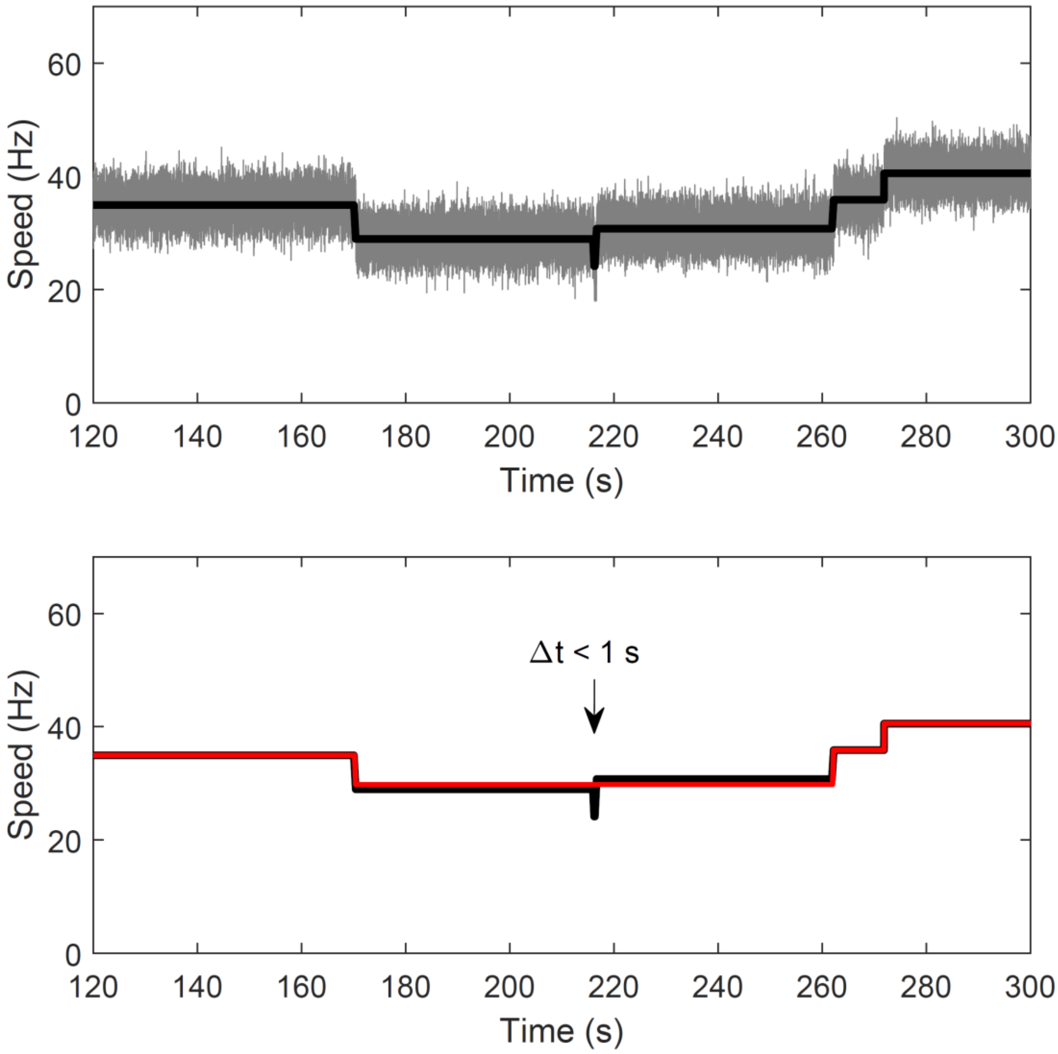

Fig. S9. An example of simulated speed trace analyzed by the step-finding algorithm. (Top) simulated speed trace (gray lines) along with the original speeds before adding noise (black lines). (Bottom) speed segments identified with the step-finding algorithms (red lines) with the parameters v_m=3 Hz, and t_m=1 s. All segments with duration longer than 1 s were correctly identified.

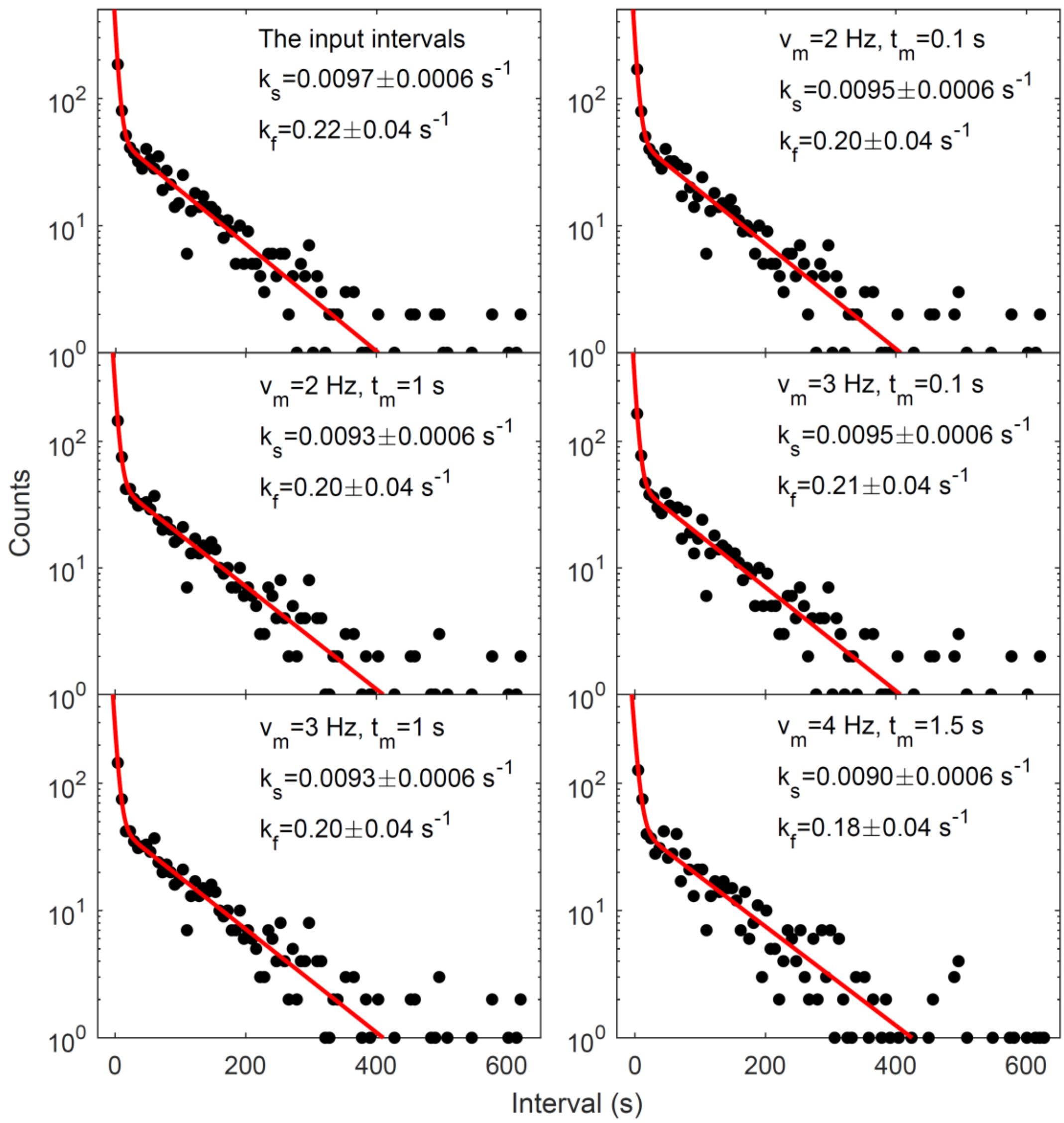

Fig. S10. The interval distributions from the simulated traces using different values of the parameters v_m and t_m in the step-finding algorithm. The red lines are fits with two-exponential shape. The fitted decay rates did not change within errors. The top-left panel is the distribution of original input intervals.