IDENTIFYING THE POWER STROKE STEP OF MYOSIN V USING A NOVEL DWELL-TIME DISTRIBUTION ANALYSIS

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Abstract

Dwell-time distributions, waiting-time distributions, and distributions of pause durations are widely reported for molecular motors based on single molecule biophysical experiments. These distributions provide important information for understanding the functional mechanism of an enzyme and its underlying kinetic and mechanical processes. We have developed a novel computational method specifically for the cyclic characteristics of molecular motors to simulate dwell-time distributions of complex kinetic schemes including branching and reverse transitions. This method, the extended absorbing boundary (EAB) method, allows global fitting of dwell-time distributions under different experimental conditions. We numerically solved ensemble average-based kinetic equations with absorbing boundary states, so that the computational result equals dwell-time distribution statistics of an infinitely long trajectory.

Using this new method, we simulated the possible mechanochemical mechanism of single-headed myosin V. By fitting the data of dwell-time distributions under different nucleotide concentrations and different directions of optical trap forces, we conclude that the power stroke is neither coupled to the Pi release step nor the ADP release step. Our analysis suggests that the power stroke is coupled to the conformational changes in the ADP-bound state.

Our computational method is widely applicable to single molecule dwell-time distributions for other molecular motors such as kinesin, RNA polymerase, helicase, and F_1 ATPase.

Background

Rief et al PNAS(2000) 97, 9482

Purcell et al PNAS(2005) 102, 13873

Dwell-time distributions
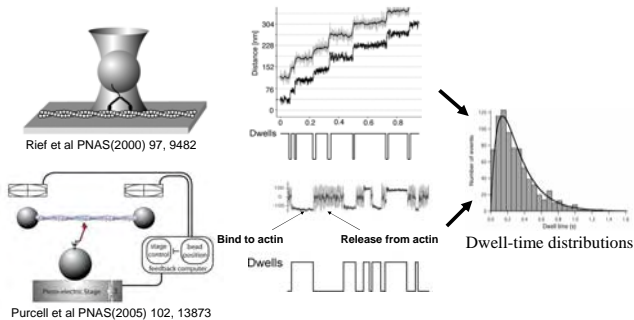
The chemical cycles of all molecular motors contain cyclic and reverse kinetic pathways, and some also have branching pathways. Therefore, there is a need to develop a method to globally fit dwell-time distributions under different experimental conditions for molecular motors with complex kinetic pathways.

Why do we need a new computational method? Disadvantages of other methods are:

1. Simple exponential formulae may misinterpret data if one does not consider reverse and branching pathways correctly.

2. Solutions obtained from the Laplace transform require the inverse Laplace transform to obtain the distribution, and the inversion can be highly nontrivial for complex kinetics.

3. Monte Carlo simulations are numerical experiments to obtain trajectories. However, to obtain high-accuracy dwell-time distributions require very long trajectory simulations.

The absorbing boundary method has been applied to obtain dwell-time distributions of problems with simple kinetic schemes. This method sets the states exiting a dwell as absorbing boundaries so that the population stayed in a dwell can be computed. Identifying the absorbing boundaries for complex kinetics with cyclic, reverse and branching pathways has never been explored. Here we developed an extended absorbing boundary (EAB) method that numerically calculates dwell-time distributions efficiently. This method is simple to implement even when the kinetic scheme is very complex.

Extended Absorbing Boundary (EAB) Method

Original kinetic scheme for myosin chemical cycle
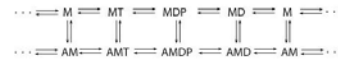
The following assumes the power stroke step is coupled to the Pi release step (AMDP→AMD).

Step 1: Identify the absorbing boundaries for dwell analysis

The absorbing boundaries are the states that exit a dwell. Since AMDP→AMD is the forward power stroke and AMD→AMDP is the backward power stroke, the kinetic scheme has to be modified so that both states serve as absorbing boundaries in the calculation.

Step 2: Determine the proportion of different states initiating a dwell

Original kinetic scheme:
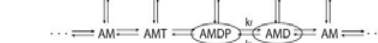
- Need to determine how many dwells start from AMDP and AMD, respectively.
- Use the original kinetic scheme to calculate the steady-state concentrations.
- Calculate the fluxes of a forward power stroke and a backward power stroke, i.e. k_f[AMDP] and k_b[AMD].
- The ratio of k_f[AMDP] and k_b[AMD] determines the proportion of initiating a dwell at AMD and AMDP states.

Step 3: Compute the derivatives of the total population of exited states

Dwell-time distributions are histograms of events binned within certain time intervals, so one should consider the number of events occurs within time intervals. In the limit, the dwell-time distributions at time t are proportional to dy/dt. In the case here, take $d([AMD']+[AMDP'])/dt$.

Comparison with Other Computational Methods

The solutions from 1. our extended absorbing boundary (EAB) method, 2. the analytical solution from the Laplace transform and 3. Monte Carlo simulation agree very well.

Histogram from Monte Carlo simulation

Solutions from our extended absorbing boundary (EAB) method and from the Laplace transform overlap almost perfectly
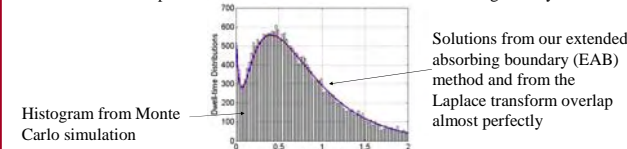
## Examine the Power Stroke Step of Myosin V

When a double-headed myosin V walks, the chemistry of two heads is likely to be regulated by strains through the necks. The front head should experience a reverse force while the rear head should experience a forward force.

Purcell, Sweeney, Spudich, PNAS(2005) 102, 13873
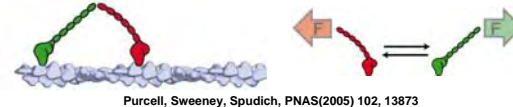
Laser trap experiments have been setup by Purcell et al to pull a single-headed myosin V in both directions to mimic these force effects. Dwell-time distributions of actin binding events have been reported from the trajectories.

Two ADP-bound Conformational States = Power Stroke

We globally fit all 6 different conditions with only one set of rate constants. The paths in the bottom show the main kinetic pathways in each case, and the rate-limiting steps (**S**).
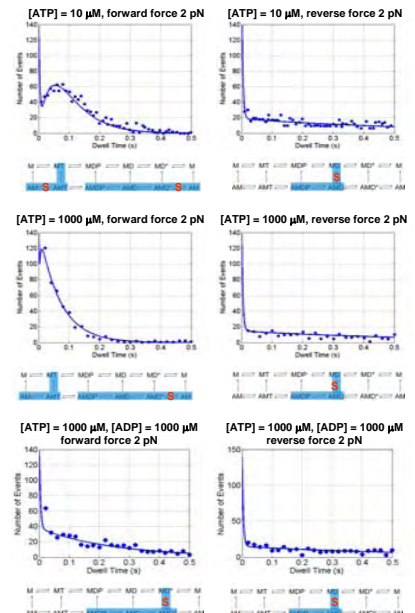
If assuming the Pi release step is the power stroke step, because this step is very fast (>250 s^{-1}), the 2 pN reverse force is not enough to slow down the kinetics to the observed low rate.

If assuming the ADP release step is the power stroke step, because this step is a rate-limiting step without force, when accelerating this step by a 2 pN forward force, one of the rate-limiting should have disappeared.

Conclusions

A computational method for dwell-time distributions, the extended absorbing boundary (EAB) method, was developed for molecular motors with cyclic, reverse and branching pathways. Using this method, we found the most probable step for myosin V to conduct the power stroke is in the ADP-bound state. A possible mechanism is that after Pi release, there are two conformational states in the ADP-bound state coupling to the power stroke.

Acknowledgements