A best-fit kinetic model for the walk of myosin V

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We model the stepping cycle of myosin V, a processive molecular motor, and develop a novel scheme to compare our results with experimental data.[1]

Myosin V is a motor protein that uses chemical energy from ATP hydrolysis to effect intracellular transport in animal cells, particularly neurones. It has two heads that bind to an actin track and a long neck region that attaches to its cargo, such as vesicles and organelles. The coordinated binding and release of the myosin heads to and from actin result in a walking motion along the track.

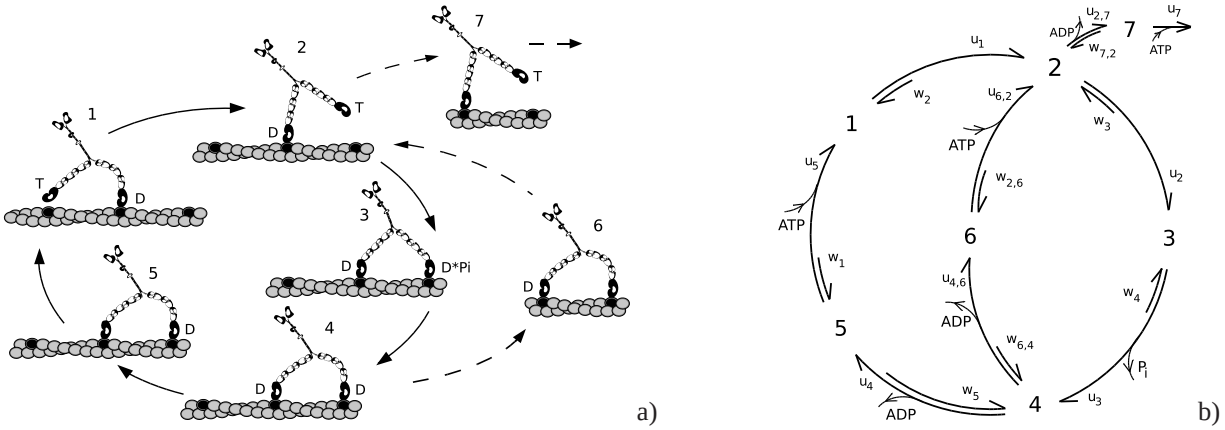

Fig. 1 Combined main and futile cycles between the seven states in the model. **a** Attachment sites spaced at 36 nm on the actin helix are shown in black. The labels T, D and $\mathrm{P_i}$ indicate where ATP, ADP and inorganic phosphate are bound to the head. (Fig. 1 of [1].) **b** Diagram showing the reaction rates, release and binding of ADP, ATP and $\mathrm{P_i}$. (Fig. 2 of [1].)

We model the myosin V walk [1] as a cycle of biochemical reactions and mechanical motions with five basic substeps (based on [2]), along with two others accounting for futile hydrolysis and detachments (Fig. 1a). The forward motion itself takes place in two stages: first it moves a distance $d_W \sim 25\mathrm{nm}$ from highly strained state 1, where the internal strain energy is E_{strain}, to unstrained state 2, and then moves a further distance $d_D \sim 11\mathrm{nm}$ by diffusion to state 3 which has internal strain bE_{strain}. We derive a set of ODEs, $\dot{\boldsymbol{P}} = \mathbb{M}\boldsymbol{P}$, where $\boldsymbol{P}(t) = (P_1, ..., P_7)$ gives the probabilities of the myosin molecule being found in each of the substates at a given time, t, and the matrix $\mathbb{M}$ contains the reaction rates (Fig. 1b)

$$
\begin{aligned}
u_1 &= \tau_{\mathrm{d}}^{-1}, & w_1 &= \tau^{-1}\mathrm{e}^{-(G_5^{\ddagger}+\Delta G_5)/k_{\mathrm{B}}T},\\
u_2 &= \tau_{\mathrm{d}}^{-1}\mathrm{e}^{-(G_2^{\ddagger}+f_{\mathrm{ex}}(d_D+\frac{1}{2}f_{\mathrm{ex}}/k_{\mathrm{H}})+bE_{\mathrm{strain}})/k_{\mathrm{B}}T}, & w_2 &= \tau_{\mathrm{d}}^{-1}\mathrm{e}^{-(E_{\mathrm{strain}}-f_{\mathrm{ex}}(d_W-\frac{1}{2}f_{\mathrm{ex}}/k_{\mathrm{H}}))/k_{\mathrm{B}}T},\\
u_3 &= \tau^{-1}\mathrm{e}^{-G_3^{\ddagger}/k_{\mathrm{B}}T}, & w_3 &= \tau_{\mathrm{d}}^{-1}\mathrm{e}^{-(G_2^{\ddagger}+\Delta G_2)/k_{\mathrm{B}}T},\\
u_4 &= \tau^{-1}\mathrm{e}^{-G_4^{\ddagger}/k_{\mathrm{B}}T}, & w_4 &= [\mathrm{P_i}]\tau^{-1}\mathrm{e}^{-(G_3^{\ddagger}+\Delta G_3-(1-b)E_{\mathrm{strain}})/k_{\mathrm{B}}T},\\
u_5 &= [\mathrm{ATP}]\tau^{-1}\mathrm{e}^{-G_5^{\ddagger}/k_{\mathrm{B}}T}, & w_5 &= [\mathrm{ADP}]\tau^{-1}\mathrm{e}^{-(G_4^{\ddagger}+\Delta G_4)/k_{\mathrm{B}}T},\\
u_{4,6} &= u_4\mathrm{e}^{-\alpha E_{\mathrm{strain}}/k_{\mathrm{B}}T}, & w_{6,4} &= w_5\mathrm{e}^{-\alpha E_{\mathrm{strain}}/k_{\mathrm{B}}T},\\
u_{6,2} &= u_5, & w_{2,6} &= w_1\mathrm{e}^{-E_{\mathrm{strain}}/k_{\mathrm{B}}T}\mathrm{e}^{-f_{\mathrm{ex}}(d_D+\frac{1}{2}f_{\mathrm{ex}}/k_{\mathrm{H}})/k_{\mathrm{B}}T},
\end{aligned}
$$

where $\tau = 10^{-8}\mathrm{s}$, $\tau_{\mathrm{d}} = 10^{-5}\mathrm{s}$, $T = 298\mathrm{K}$, k_{B} is the Boltzmann constant, $G_i^{\ddagger}$ is the chemical energy barrier between state i and its forward neighbour, ΔG_i is the energy difference between the two states, f_{ex} is the component of the external force parallel to actin, k_{H} is the effective Hookeian spring constant and $\alpha E_{\mathrm{strain}}$ is the strain that must be overcome in order to release or bind ADP from or to the front head. These equations can be solved to give the myosin velocity, run length (distance travelled before detachment) and dwell time (average delay before a forward step) as a function of the external force and the concentrations of nucleotides for comparison with experimental data.

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Our model has nine undetermined parameters: E_{strain}, $\alpha E_{\mathrm{strain}}$, $G_2^{\ddagger}$, $G_3^{\ddagger}$, $G_4^{\ddagger}$, $G_5^{\ddagger}$, ΔG_2, ΔG_3 and ΔG_4. We searched [1] a $(25 k_{\mathrm{B}}T)^9$ parameter space for the best fit with experiment by defining a cost function, $\Delta = \sum_{i=1}^{17} \Delta^{(i)}$, to quantify the agreement between model and data. Each $\Delta^{(i)}$ measures the difference between the model prediction and an experimental data point, e.g. $\Delta^{(1)} = [V - 540\,\mathrm{nm/s}]^2/(54\,\mathrm{nm/s})^2$, where V is the predicted velocity, 540 nm/s is an experimental measurement and the variance, $(54\,\mathrm{nm/s})^2$, describes the acceptable deviation from the target value. We first evaluated the cost function at 50 million points spread evenly in the parameter space using the Sobol quasi-random sequence and then passed the 50 points with the lowest values to a simulated annealing routine where the cost function was the energy term.

The best-fit solution [1] has one rate-limiting step: the release of ADP from the rear head in state 4. There is a trade-off here as slowing ADP release reduces the flux around the futile cycle and detachments, but also slows velocity. There were no optimal solutions with a different or with more than one rate-limiting step. We found a reasonable fit with data (Fig. 2), but were not able to reproduce the trend of run length with varying ADP concentration, which suggests something may be missing or overlooked in the current models of the myosin V walk and that further experimental investigation may be needed.

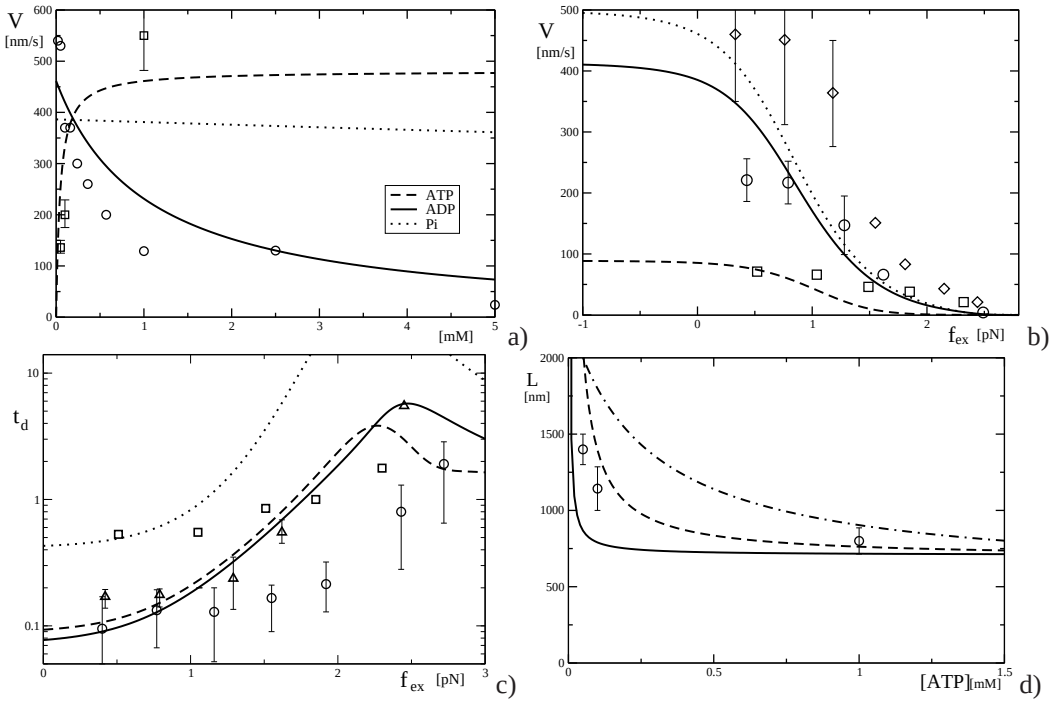

Fig. 2 Model predictions (lines) compared with experimental data (symbols). Where not specified: [ATP]=1 mM, [ADP] = 0.1 μM, [P_i] = 0.1 μM. **a** Velocity against nucleotide concentration with data from [3]. (Fig. 5 of [1].) **b** Velocity against external force with data from [4]: [ATP]=1 mM and [ADP]=200 μM (circles), [ATP]=1 mM and [ADP]=1 μM (squares) and [ATP]=10 μM and [ADP]=1 μM (diamonds). (Fig. 6 of [1].) **c** Dwell time against external force with data from [5] (circles: [ATP]=2 mM) and [4] (squares: [ATP]=10 μM; triangles: [ATP]=1 mM and [ADP]=200 μM). (Fig. 7 of [1].) **d** Run length against ATP concentraion with data from [3]: [ADP]=1 mM (dotted-dashed line), 100 μM (dashed line) and 10 μM (solid line). (Fig. 9 of [1].)

We believe ours was the first attempt [1] to explore systematically whether a given model of a molecular motor reaction cycle, with unknown parameters, is capable of reproducing observed experimental trends for best-fit values of those parameters. This new approach could be used in future to pinpoint where existing models fail or need modification, and to differentiate between competing theoretical models according to their fit with experimental data. The ultimate aim is to clarify the underlying mechanisms of molecular motor stepping cycles where these are still not precisely understood.

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