Chemomechanical coupling and motor cycles of myosin V
Supporting Material

Veronika Bierbaum and Reinhard Lipowsky
Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces,
14424 Potsdam, Germany

S1 The chemomechanical cycle $\mathcal{F}$

In this section, we outline the considerations that lead to the chemomechanical step cycle $\mathcal{F}$.
Because of the high binding affinity of ATP to myosin (1), the motor heads are, for saturating concentrations of ATP, very likely to attain the T state before binding to the filament. As the motor is strongly bound to the filament both in the E and D states but only weakly bound in the T state (1), and ATP hydrolysis is fast compared to ADP release, the motor will end up in the states DE, ED, or DD when binding to the filament to start its processive run.
Experiments indicate that the molecule dwells in the DD state for most of the time between two succesive steps (2, 3). Starting from this state, the release of ADP can lead to two states, ED or DE. For two-headed myosin constructs, the rate of ADP release in the myosin motor has been investigated extensively and is thought to differ for the rear and the leading head of the motor (4, 5). The different experiments present highly diverse data, but agree that the rate of ADP release for the front head is much slower than for the rear head. This observation is taken into account by omitting the state DE from the network.
In the state ED, both heads are strongly bound to the filament. The binding of an ATP molecule to the rear head leads to the TD state, which, through weakening of the actomyosin bond, is followed by a mechanical step. This results in an interchange of the two heads and in the state DT, and subsequent hydrolysis leads to the state DD and the completion of the forward stepping cycle $\mathcal{F}$.

S2 Cyclic fluxes and balance conditions

The entropy that is produced during the revolution of a cycle can be readily connected to the work performed by the motor. For each dicycle $\mathcal{C}^d_\nu$, the average entropy ΔS produced during one dicycle completion is given by (6–8)

$$\Delta S(\mathcal{C}^d_\nu) = k_\mathrm{B} \ln \prod_{|ij\rangle}^{\nu,d} \left(\frac{\omega_{ij}}{\omega_{ji}} \right) . \tag{S.1}$$

In the steady state, the internal energy, which arises from the chemical reaction and mechanical work during the processive motion of the motor, does not change on a cycle $\mathcal{C}^d_\nu$ of the network,

$$\Delta U(\mathcal{C}^d_\nu) = 0. \tag{S.2}$$

The first law of thermodynamics together with the heat $\Delta Q(\mathcal{C}^d_\nu) = T\Delta S(\mathcal{C}^d_\nu)$ released by the motor during one completion of the dicycle $\mathcal{C}^d_\nu$ leads to a balance condition for this dicycle as given by

$$k_\mathrm{B} T \ln \prod_{|ij\rangle}^{\nu,d} \left(\frac{\omega_{ij}}{\omega_{ji}} \right) = E_\mathrm{ch}(\mathcal{C}^d_\nu) - W_\mathrm{me}(\mathcal{C}^d_\nu) \tag{S.3}$$

where $E_{\rm ch}(\mathcal{C}^d_\nu)$ and $W_{\rm me}(\mathcal{C}^d_\nu)$ are the chemical energy supplied to the motor and the mechanical work performed by the motor, respectively, during one completion of dicycle $\mathcal{C}^d_\nu$. The chemical energy $E_{\rm ch}(\mathcal{C}^d_\nu)$ can be expressed in terms of the reaction free enthalpy $\Delta\mu$ that arises from addition of one ATP molecule to the system and the subsequent release of one ADP and phosphate, which is, for dilute solutions, given by

$$\Delta\mu = k_{\rm B}T \ln\left(K_{\rm eq}\,\frac{[\mathrm{ATP}]}{[\mathrm{ADP}][\mathrm{P}]}\right), \tag{S.4}$$

with $K_{\rm eq} \simeq 4.9\times 10^{11}\mu$M being the equilibrium constant of the reaction. Thus, if the dicycle $\mathcal{C}^d_\nu$ contains $n_{\rm h}(\mathcal{C}^d_\nu)$ ATP hydrolysis and $n_{\rm s}(\mathcal{C}^d_\nu)$ ATP synthesis transitions, the chemical energy has the form

$$E_{\rm ch}(\mathcal{C}^d_\nu) = \Big(n_{\rm h}(\mathcal{C}^d_\nu) - n_{\rm s}(\mathcal{C}^d_\nu)\Big)\,\Delta\mu\,. \tag{S.5}$$

Now, consider all transitions $|ij\rangle$ of the dicycle $\mathcal{C}^d_\nu$ and denote the corresponding mechanical displacements by ℓ_{ij}. The total mechanical displacement after the completion of $\mathcal{C}^d_\nu$ is then given by

$$\ell(\mathcal{C}^d_\nu) = \sum_{|ij\rangle}^{\nu,d} \ell_{ij}\,. \tag{S.6}$$

If the dicycle $\mathcal{C}^d_\nu$ contains more than one transition $|ij\rangle$ with $\ell_{ij}\neq 0$, the total mechanical step of size $\ell(\mathcal{C}^d_\nu)$ consists of several substeps. Furthermore, if the motor moves against the resisting force F, it performs the mechanical work (7)

$$W_{\rm me}(\mathcal{C}^d_\nu) = F\,\ell(\mathcal{C}^d_\nu) = F\sum_{|ij\rangle}^{\nu,d}\ell_{ij} \tag{S.7}$$

during one completion of dicycle $\mathcal{C}^d_\nu$.

Using the decomposition $\omega_{ij} = \omega_{ij,0}\,\Phi(F)$ of the transition rates as given by Eq. (3) in the main text, the balance condition (S.3) can be decomposed into two conditions, which have the general form (6, 7)

$$k_{\rm B}T\ln\prod_{|ij\rangle}^{\nu,d}\left(\frac{\omega_{ij,0}}{\omega_{ji,0}}\right) = E_{\rm ch}(\mathcal{C}^d_\nu) = \Big(n_{\rm h}(\mathcal{C}^d_\nu) - n_{\rm s}(\mathcal{C}^d_\nu)\Big)\,\Delta\mu \tag{S.8}$$

and

$$k_{\rm B}T\ln\prod_{|ij\rangle}^{\nu,d}\left(\frac{\Phi_{ij}}{\Phi_{ji}}\right) = -W_{\rm me}(\mathcal{C}^d_\nu) = -F\,\ell(\mathcal{C}^d_\nu)\,. \tag{S.9}$$

Such a decomposition is always possible since the reaction free enthalpy $\Delta\mu$ and the load force F are two independent control parameters. We use the convention that negative values of F correspond to assisting forces, which act in the preferred direction of forward stepping, i. e., towards the plus-end of the actin filament, while resisting forces are described by positive values of F.

We now apply the general balance conditions (S.8) and (S.9) to the chemomechanical network for myosin V as studied here, see Fig. 2 in the main text. In the absence of a load force, i.e., for $F=0$, the transition rates $\omega_{ij} = \omega_{ij,0}$ have the form $\omega_{ij,0} = \hat\kappa_{ij}[X]$ for X-binding during the transition $|ij\rangle$ and $\omega_{ij,0} = \kappa_{ij}$ for X-release during $|ij\rangle$, see Eq. (4) of the main text. For the cycles $\mathcal{F}$ and $\mathcal{E}$, the zero-force balance condition (S.8) is then equivalent to

$$K_{\rm eq} = \frac{\kappa_{12}\hat\kappa_{23}\kappa_{34'}\kappa_{41}}{\hat\kappa_{21}\kappa_{32}\kappa_{4'3}\hat\kappa_{14}} = \frac{\kappa_{25}\hat\kappa_{56}\kappa_{62}}{\hat\kappa_{52}\kappa_{65}\hat\kappa_{26}} \quad \text{for} \quad F=0\,. \tag{S.10}$$

Since the ratchet or mechanical slip cycle $\mathcal{M}$ does not contain any chemical transition, we also have

$$\kappa_{55'} = \kappa_{5'5} \quad \text{for } F = 0\,, \tag{S.11}$$

which reflects the fact that there is a priori no preferred direction for the motor to perform a forward or a backward step in the state EE.

In the presence of a load force, i.e., for $F \neq 0$, the force-dependent balance condition (S.9) leads to

$$\frac{\Phi_{12}\Phi_{23}\Phi_{34'}\Phi_{41}}{\Phi_{21}\Phi_{32}\Phi_{4'3}\Phi_{14}} = \exp\left(-\frac{\ell}{k_{\mathrm{B}}T}F\right) \quad \text{for dicycle } \mathcal{F}^{+} \tag{S.12}$$

with $\ell \equiv \ell(\mathcal{F}^{+})$ and to

$$\frac{\Phi_{25}\Phi_{56}\Phi_{62}}{\Phi_{52}\Phi_{65}\Phi_{26}} = 1 \quad \text{for dicycle } \mathcal{E}^{+} \tag{S.13}$$

as follows from $W_{\mathrm{me}}(\mathcal{E}^{+}) = 0$ since the enzymatic cycle $\mathcal{E}$ does not contain any mechanical transition $|ij\rangle$ with $\ell_{ij} \neq 0$.

The balance condition (S.12) for $\mathcal{F}^{+}$ can be further simplified if the transition rates for the mechanical transitions $|34'\rangle$ and $|4'3\rangle$ are taken to have the form as given by Eqs. (7) and (8) in the main text. This form implies

$$\frac{\Phi_{34'}(F)}{\Phi_{4'3}(F)} = \exp\left(-\frac{\ell}{k_{\mathrm{B}}T}F\right). \tag{S.14}$$

When this relation is combined with the balance condition (S.12), we obtain

$$\frac{\Phi_{12}\Phi_{23}\Phi_{41}}{\Phi_{21}\Phi_{32}\Phi_{14}} = 1\,. \tag{S.15}$$

As shown in Ref. (7), the relation (S.14) can also be derived from a local form of the balance condition if one assumes that the change in free energy arising from the force F is independent of the motor state. The latter assumption is valid in the limit of small F but need not apply to arbitrary values of F. For the mechanical slip cycle $\mathcal{M}$, on the other hand, the balance condition (S.12) is equivalent to

$$\frac{\Phi_{55'}(F)}{\Phi_{5'5}(F)} = \exp\left(-\frac{\ell}{k_{\mathrm{B}}T}F\right) \tag{S.16}$$

as confirmed by the explicit calculation in the next subsection S3.

S3 Functional form of mechanical stepping rates

To obtain stepping rates valid for a large range of load forces, we use, as discussed in (9), a coarse graining approach that is based on the discretization of the continuous Fokker-Planck-equation. The stepping rates calculated in this way automatically fulfill the correct balance condition. A different method based on calculations of the mean first passage time can be found in (10, 11). We start from a Fokker-Planck-equation for the diffusion of a particle over a potential $V(x)$ along a continuous variable x. For simplicity, we take a sawtooth potential $U(x)$ with period ℓ, height U_{ba}, and slope U_{ba}/ℓ, and impose periodic boundary conditions on the potential. The simplest discretization of the continuous equation consists in assigning lattice sites to the potential in two successive minima, i. e., at locations $x_n = n\ell$ and $x_{n+1} = (n+1)\ell$ for integer n, as indicated in Fig. S1. $U(x)$ is then given by

$$U(x) = -\frac{U_{\mathrm{ba}}}{\ell}(x - n\ell) \quad \text{for} \quad n\ell \leq x < (n+1)\ell \tag{S.17}$$

The transition rates between two neighbouring sites x_n and x_{n+1} are calculated as

$$\omega_{n,\ n+1} = \frac{e(x_n,\ x_{n+1})}{\mathcal{E}(x_n,\ n_{k+1})\ell_\Omega}, \tag{S.18}$$

$$\omega_{n+1,\ n} = \frac{1}{\mathcal{E}(x_n, x_{n+1})\ell_\Omega}, \tag{S.19}$$

as given in Eq. 3.9 of (9), with

$$e(x,y) = \exp\left(\frac{1}{k_\mathrm{B}T}(x-y)F\right) \tag{S.20}$$

and

$$\mathcal{E}(x,y) = 1/D \int_x^y dz\ \exp(V(z) - V(y)), \tag{S.21}$$

where D is a diffusion constant, ℓ_Ω a localization and $V(x) = \frac{1}{k_\mathrm{B}T}\left(U(x) + Fx\right)$ is the forced potential, with $F > 0$ being a backward force. Note that $\mathcal{E}(x,y)$ holds for $x < y$, with both x and y being from one interval of the potential. For the sawtooth potential as given by S.17, the forward and the backward transitions have the form

$$\begin{aligned} \omega_{n,\ n+1} &= \frac{D}{k_\mathrm{B}T} \cdot \frac{F\ell - U_\mathrm{ba}}{\ell^2} \cdot \frac{\exp\left[-\frac{\ell}{k_\mathrm{B}T}F\right]}{1 - \exp\left[\frac{1}{k_\mathrm{B}T}(U_\mathrm{ba} - F\ell)\right]}, & \text{(S.22)} \\ \omega_{n+1,\ n} &= \frac{D}{k_\mathrm{B}T} \cdot \frac{F\ell - U_\mathrm{ba}}{\ell^2} \cdot \frac{1}{1 - \exp\left[\frac{1}{k_\mathrm{B}T}(U_\mathrm{ba} - F\ell)\right]} & \text{(S.23)} \\ & & \text{(S.24)} \end{aligned}$$

where we have set $\ell_\Omega = \ell$. The rates automatically obey the requirement that $\frac{\omega_{n,\ n+1}}{\omega_{n+1,\ n}} = \exp(-\frac{\ell}{k_\mathrm{B}T}F)$. In this way, a backward step in the ratchet has the rate $\omega_{55'} = \omega_{n,\ n+1}$ and a forward step is given by $\omega_{5'5} = \omega_{n+1,\ n}$. By definition, we then have $\kappa_{55'} = \omega_{55'}(F = 0)$ and $\kappa_{5'5} = \omega_{5'5}(F = 0)$, respectively.

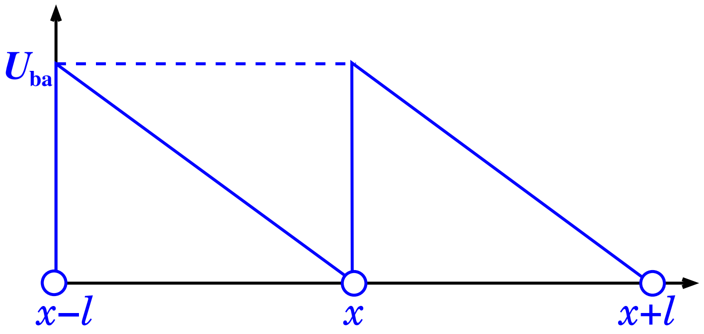

Figure S 1: Periodic sawtooth potential with slope $-U_\mathrm{ba}/\ell$, barrier height U_ba and periodicity ℓ.

S4 Balance condition for phosphate binding

For the chemomechanical cycle $\mathcal{F}$, the zero-force balance condition (S.10) leads to

$$\hat{\kappa}_{14} = \frac{\hat{\kappa}_{23}\kappa_{34'}\kappa_{41}\kappa_{12}}{\hat{\kappa}_{21}\kappa_{4'3}\kappa_{32}K_{\mathrm{eq}}}, \tag{S.25}$$

whereas it implies

$$\hat{\kappa}_{26} = \frac{\kappa_{25}\hat{\kappa}_{56}\kappa_{62}}{\hat{\kappa}_{52}\kappa_{65}K_{\mathrm{eq}}}. \tag{S.26}$$

for the enzymatic slip cycle $\mathcal{E}$. It follows directly from these conditions that $\hat{\kappa}_{14} \neq \hat{\kappa}_{26}$. The latter inequality reflects the differences in the chemical transitions $|14\rangle$ and $|26\rangle$. First, the phosphate is released from the leading head during $|14\rangle$ but from the trailing head during $|26\rangle$. Second, the second head is in the D state during $|14\rangle$ but in the E state during $|26\rangle$. Inspection of Eq. (S.25) and (S.26) shows that the rates of phosphate binding in the cycles $\mathcal{F}$ and $\mathcal{E}$, $\hat{\kappa}_{14}$ and $\hat{\kappa}_{26}$, are proportional to each other and satisfy the relation

$$\hat{\kappa}_{14} = \hat{\kappa}_{26}\,\frac{\kappa_{34'}}{\kappa_{4'3}}. \tag{S.27}$$

S5 Table of parameters for force-dependence

Force dependence	Transition rate ω_{ij}	Parameter	Value
Fueled stepping	$\omega_{34'},\ \omega_{4'3}$	θ	0.65
Forced stepping	$\omega_{55'},\ \omega_{5'5}$	U_{ba} D	$20\ k_{\mathrm{B}}T$ $4.7 \cdot 10^2\ \ \mathrm{nm}^2/\mathrm{s}$
ATP binding and release, $\mathcal{F}$	$\omega_{23},\ \omega_{32}$	$\chi_{23},\ \chi_{32}$	0
ATP binding, $\mathcal{E}$	ω_{56}	$\chi_{56},\ F'$	4, 1.6 pN
ATP release, $\mathcal{E}$	ω_{65}	χ_{65}	0
ADP binding and release, $\mathcal{F}$	$\omega_{12},\ \omega_{21}$	$\chi_{12},\ \chi_{21}$	0
ADP binding, $\mathcal{E}$	ω_{52}	χ_{52}, F'	4, 1.6 pN
ADP release, $\mathcal{E}$	ω_{25}	χ_{25}	0
P binding and release, $\mathcal{F}$ and $\mathcal{E}$	$\omega_{14},\ \omega_{26},\ \omega_{41},\ \omega_{62}$	$\chi_{14},\ \chi_{26},\ \chi_{41},\ \chi_{62}$	0

Table S 1: Parameters for the force depencence of the mechanical transitions and the ATP binding and release rate. The corresponding functions are specified in the main article.

S6 Velocity as a function of [ATP] for high assisting and resisting loads

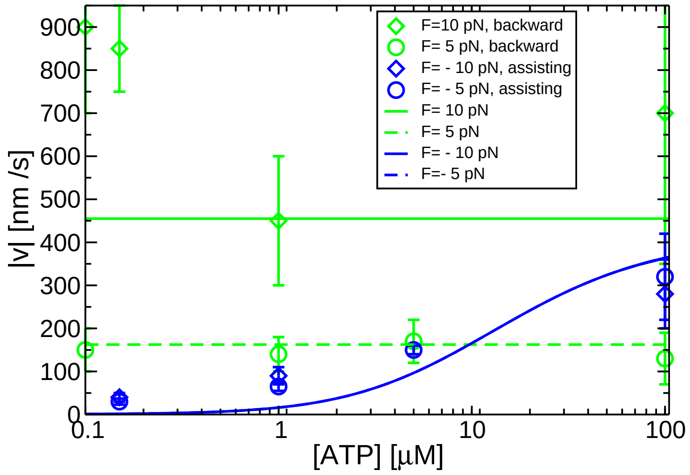

Figure S 2: Ratcheting behaviour as measured in (13) (symbols) compared to the theoretical results (lines). The figure shows the absolute value of the motor velocity $|v|$, as a function of [ATP] for superstall forces $F = \pm 5$ and ± 10 pN. The blue symbols and lines correspond to assisting loads, and the green ones to backward loads. The circles are for $F = \pm 5$pN, diamonds for $F = \pm 10$ pN. For backward loads, our theory leads to velocities that do not depend on [ATP]. For 5 pN backward load, the velocity (green dashed line) is in good agreement with the data, while for 10 pN pull, the theoretical velocity is lower than the experimental one. In the case of forward forces, the theoretical velocity matches the data qualitatively. Note that the blue dashed and the solid line are identical. For very low values of [ATP], the calculated velocity is underestimated, which might be due to the fact that (13) report, even in the absence of an external load, considerably higher stepping velocities at low [ATP] compared to other groups (12, 14).

References

1. de La Cruz, E., A. M. Wells, S. S. Rosenfeld, M. E. Ostap, and H. L. Sweeney, 1999. The kinetic mechanism of myosin V. *Proceedings of the National Academy of Sciences of the United States of America* 96:13726.
2. Rief, M., R. S. Rock, A. D. Mehta, M. S. Mooseker, R. E. Cheney, and J. A. Spudich, 2000. Myosin-V stepping kinetics: A molecular model for processivity. *Proceedings of the National Academy of Sciences of the United States of America* 97:9482.
3. Veigel, C., F. Wang, M. L. Bartoo, J. R. Sellers, and J. E. Molloy, 2002. The gated gait of the processive molecular motor, myosin V. *Nature cell biology* 4:59.
4. Rosenfeld, S. S., and H. L. Sweeney, 2004. A model of myosin V processivity. *The Journal of biological chemistry* 279:40100.
5. Forgacs, E., S. Cartwright, T. Sakamoto, J. R. Sellers, J. E. T. Corrie, M. R. Webb, and H. D. White, 2008. Kinetics of ADP dissociation from the trail actomyosin V following the power stroke. *J. Biol. Chem.* 283:766.
6. Liepelt, S., and R. Lipowsky, 2007. Steady-state balance conditions for molecular motor cycles and stochastic nonequilibrium processes. *EPL* 77:6.
7. Lipowsky, R., and S. Liepelt, 2008. Chemomechanical Coupling of Molecular Motors: Thermodynamics, Network Representations, and Balance Conditions. *Journal of Statistical Physics* 130:39. Erratum: *Journal of Statistical Physics*, 135:777, 2009.
8. Lipowsky, R., S. Liepelt, and A. Valleriani, 2009. Energy conversion by molecular motors coupled to nucleotide hydrolysis. *Journal of Statistical Physics* 135:951.
9. Lipowsky, R., and N. Jaster, 2003. Molecular motor cycles: From ratchets to networks. *J. Stat. Phys.* 110:1141.
10. Gardiner, C., 2009. Handbook of Stochastic Methods: a handbook for the natural and social sciences. Springer.
11. Zwanzig, R., 2001. Nonequilibrium Statistical Mechanics. Oxford University Press.
12. Komori, Y., and A. H. Iwane, 2007. Myosin-V makes two Brownian 90 degrees rotations per 36-nm step. *Nature structural & molecular biology* 14:968.
13. Gebhardt, J., A. E.-M. Clemen, J. Jaud, and M. Rief, 2006. Myosin-V is a mechanical ratchet. *Proceedings of the National Academy of Sciences of the United States of America* 103:8680.
14. Forkey, J. E., M. E. Quinian, M. A. Shaw, J. E. T. Corrie, and Y. E. Goldman, 2003. Three-dimensional structural dynamics of myosin V by single-molecule fluorescence polarization. *Nature* 422:399.