Fitted parameters for the four-state models for kinesin yielding the velocity and randomness plots shown in Fig. 7

The parameters are as follows: First, requiring that the biochemical states (1), (2) and (3) be colocalized and fixing the substep distance at $d_0 = 0.15$ nm (equal to that for $N = 2$) yields satisfactory fits to both $V(F_x, [\mathrm{ATP}])$ and $r(F_x, [\mathrm{ATP}])$ (see the solid curves in Fig. 7) when the zero-load rate constants are given by

$$\begin{aligned} k_0^0 &= 1.45\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad w_1^0 = 80\ \mathrm{s}^{-1}, \qquad u_1^0 = 520\ \mathrm{s}^{-1}, \\ w_2^0 &= 20\ \mathrm{s}^{-1}, \qquad u_2^0 = 250\ \mathrm{s}^{-1}, \qquad w_3^0 = 20\ \mathrm{s}^{-1}, \\ u_3^0 &= 250\ \mathrm{s}^{-1}, \qquad k_0' = 0.03\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad c_0 = 0.5\ \mu\mathrm{M}, \end{aligned} \qquad [\mathbf{11}]$$

while the load distribution vectors, apart from $\boldsymbol{\theta}_1^+ = \boldsymbol{\theta}_2^\pm = \boldsymbol{\theta}_3^- = 0$, are (with the factor $c_\parallel = 1.45$ incorporated in the z components)

$$\begin{aligned} \boldsymbol{\theta}_0^+ d &= (1.15, 0, -0.35)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_1^- d = (-1.00, 0, -0.10)\ \mathrm{nm}, \\ \boldsymbol{\theta}_3^+ d &= (0.42, 0, -0.35)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_0^- d = (7.63, 0, 0.80)\ \mathrm{nm}. \end{aligned} \qquad [\mathbf{12}]$$

The forward rates in **11** are similar to those found previously (see ref. 1 Eq. **14**); but the balance of the reverse rates is rather different as, of course, are the load distribution factors.

Next, imposing $\boldsymbol{\theta}_1^+ = \boldsymbol{\theta}_2^- = \boldsymbol{\theta}_3^+ = \boldsymbol{\theta}_0^- = 0$, which implies that states (1), (2) and (3), (4) are colocalized, and fixing $d_0 = 0.6$ nm yields the fit

$$\begin{aligned} k_0^0 &= 1.3\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad w_1^0 = 20\ \mathrm{s}^{-1}, \qquad u_1^0 = 290\ \mathrm{s}^{-1}, \\ w_2^0 &= 40\ \mathrm{s}^{-1}, \qquad u_2^0 = 290\ \mathrm{s}^{-1}, \qquad w_3^0 = 0.9\ \mathrm{s}^{-1}, \\ u_3^0 &= 780\ \mathrm{s}^{-1}, \qquad k_0' = 7\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad c_0 = 70\ \mu\mathrm{M}, \end{aligned} \qquad [\mathbf{13}]$$

with the load distribution vectors (including the factor $c_\parallel$)

$$\begin{aligned} \boldsymbol{\theta}_0^+ d &= (0.90, 0, -0.25)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_1^- d = (-0.29, 0, -0.57)\ \mathrm{nm}, \\ \boldsymbol{\theta}_2^+ d &= (0.29, 0, -0.32)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_3^- d = (7.30, 0, 1.14)\ \mathrm{nm}. \end{aligned} \qquad [\mathbf{14}]$$

See the dashed curves in Fig. 7.

Finally, when we impose the colocalization of states (2), (3) and (4), by setting $\boldsymbol{\theta}_2^+ = \boldsymbol{\theta}_3^\pm = \boldsymbol{\theta}_0^- = 0$, and again take $d_0 = 0.6$ nm, the data are best fitted (see the dotted curves in Fig. 7) with the parameters

$$\begin{aligned} k_0^0 &= 1.65\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad w_1^0 = 4.0\ \mathrm{s}^{-1}, \qquad u_1^0 = 290\ \mathrm{s}^{-1}, \\ w_2^0 &= 5\ \mathrm{s}^{-1}, \qquad u_2^0 = 1800\ \mathrm{s}^{-1}, \qquad w_3^0 = 20\ \mathrm{s}^{-1}, \\ u_3^0 &= 300\ \mathrm{s}^{-1}, \qquad k_0' = 55\ \mu\mathrm{M}^{-1}\cdot\mathrm{s}^{-1}, \qquad c_0 = 1.0\ \mu\mathrm{M}, \end{aligned} \qquad [\mathbf{15}]$$

and the load distribution vectors

$$\begin{aligned} \boldsymbol{\theta}_0^+ d &= (0.94, 0, -0.37)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_1^- d = (-0.33, 0, -0.54)\ \mathrm{nm}, \\ \boldsymbol{\theta}_1^+ d &= (-0.16, 0, -0.14)\ \mathrm{nm}, \qquad \boldsymbol{\theta}_2^- d = (7.75, 0, 1.05)\ \mathrm{nm}. \end{aligned} \qquad [\mathbf{16}]$$

This fit is considerably improved if one allows $d_0 = 1.1$ nm; but, as explained, such a relatively large value is excluded by the single-step observations (2,3).

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