Chapter 4.

Consequences of neck linker elasticity on kinesin motion along microtubules

The models of kinesin function presented in several of our publications [1,2 3] treat the neck linker as a key element of the mechanism but do not include its elasticity in detail since we used the strictly overdamped limit. In this note the elasticity of the neck linker is considered in order to see how it affects the conclusions drawn for the simpler models and to account for the secondary relaxation it introduces.

The key issue is the rate of relaxation. For the case of a passive neck linker the relaxation time for the Brownian motion of the kinesin head is about a picosecond. The system operates at very low Reynolds number which means viscosity dominates inertia. Once the neck linker binding to the bound kinesin head's β-sheet edge is completed the lack of inertia prevents the unbound kinesin head from further forward ballistic motion. However, if the neck linker possesses elasticity, the relaxation process developes a secondary relaxation that operates on a time scale longer than a picosecond. How much longer is the issue. For a sufficiently long secondary relaxation, the free kinesin head might make it all the way to the new binding site after all. As we shall see, the physical parameters for this process produce a secondary relaxation time that while longer than a picosecond is nevertheless still short enough for the conclusions based on the earlier models to still hold true. For nomenclature and diagrams see [1,2,3].

The neck linker is typically a polypeptide containing 13-15 amino acids in an unstructured conformation. Only when it forms the outer edge of the kinesin head central β-sheet does it develop a rigid conformation. However the other neck linker attached to the free kinesin head remains unstructured. It is this other neck linker that is of interest. The bound neck linker is directly attached to the neck itself. The neck has a mass of about 500-600 kd, compared to the mass of the head that is about 50 kd. So the neck linker docking to the bound head pulls the neck forward, and this in turn pulls the free head forward through the intermediary neck

linker. It is known that the neck linker docking involves an energy of a few $k_B T$ over a distance of a few nm. The effective "secular" force is of order

$$F_S = \frac{4 \times 10^{-14}}{10^{-7}} \frac{erg}{cm} = 4\, pN$$

This is not much force and will produce a velocity for the neck that is determined by the viscous drag on the neck. The precise value for the drag on the neck can not be calculated analytically due to the long coiled coil shape of the neck. However, for the head which is globular and has a mass of 8 x 10^{-20} gm and can be approximated by a sphere the drag, α, is known to be about 8 x 10^{-8} gm/s. Surely the drag on the neck will be larger but not by too much since an equivalent sphere with 10 times the mass of the head would have a radius only $\sqrt[3]{10}$ times as big. Thus we will place an upper bound on the velocity of the neck at the moment neck linker docking ceases given by using just the drag, α, on the head.

$$v_S = \frac{F_S}{\alpha} = \frac{4}{8 \times 10^{-8}} \frac{pN}{gm/s} = 5\, cm/s$$

Recall (see p.6 of chapter 2) that for the low Reynolds number the distance traveled once the force stops and the neck is moving at 5 cm/s is given by the product of this velocity and the relaxation time. For a relaxation time of one picosecond the result is much less than an angstrom by almost four orders of magnitude. With a proper value for the neck's drag this result should be at least 2 or 3 times smaller.

The description of the motion of the free kinesin head when the neck linker elasticity is included is that of the Brownian motion of a harmonic oscillator in the limit of modest extensions. Measurements using atomic force microscopy (AFM) and laser tweezers have lead to a formula for the force, F, versus relative extension, x/L, where x is the extension and L is the contour length in a worm-like-chain model for an unstructered polypeptide, given by [4]

$$FP = kT \left[\frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} - \frac{1}{4} + \frac{x}{L} \right]$$

in which P is the persistence length. If the contour length, L, is made up from N links of length a then $L = Na$ and Kuhn's formula says $a = 2P$. In the linear regime for the extension we have the approximation

$$\frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} - \frac{1}{4} + \frac{x}{L} \cong \frac{1}{4}\left(1 + 2\frac{x}{L}\right) - \frac{1}{4} + \frac{x}{L} = \frac{3x}{2L}$$

Inserting this into the force formula above and dividing by P yields the Hooke's law approximation for the Hooke constant K:

$$F \cong \frac{3k_B T}{aL}x = Kx$$

Even a relative extension of 0.5 gives an approximate force that isn't yet 2 times too small compared to the complete formula. The freely-jointed-chain model yields the same linear result [3].

The langevin equation for the motion of the free head's center of mass is given by

$$M\frac{d}{dt}v = -\alpha v + \tilde{F} + K(X - x)$$

in which M is the mass of the kinesin head, $\tilde{F}$ is the Brownian force, X is the position of the neck's attachment to the free neck linker and $X - x$ is now the extension of the free neck linker relative to the neck. The extension satisfies

$$\frac{d}{dt}x = v$$

Instead of a one dimensional Langevin equation we have a two dimensional equation when there is an elastic restoring force. It is useful to switch to the variable y defined by

$$y = \omega x$$

in which the frequency ω is defined according to the Hooke formula

$$K \equiv M\omega^2$$

The coupled equations can now be rendered in the form

$$\frac{d}{dt}\begin{pmatrix} y \\ v \end{pmatrix} = \begin{pmatrix} 0 & \omega \\ -\omega & -\alpha/M \end{pmatrix}\begin{pmatrix} y \\ v \end{pmatrix} + \begin{pmatrix} 0 \\ (\tilde{F} + KX)/M \end{pmatrix}$$

From here on we will ignore the fluctuating force and only look at the average behavior.

This harmonic oscillator approximation can be solved analytically. Incorporation of the complete nonlinear force formula above requires numerical simulations. Much can be learned from the harmonic case, nevertheless. Introduce the coupling matrix, G, defined by

$$G = \begin{pmatrix} 0 & -\omega \\ \omega & \alpha/M \end{pmatrix}$$

The formal solution to the two component Langevin equation is

$$\begin{pmatrix} y(t) \\ v(t) \end{pmatrix} = e^{-Gt}\begin{pmatrix} y(0) \\ v(0) \end{pmatrix} + \int_0^t ds\ e^{-G(t-s)}\begin{pmatrix} 0 \\ KX(s)/M \end{pmatrix}$$

We want to apply this result for times after the neck linker docking has just been completed. Thus $v(0)$ is expected to be a few cm/s as discussed above, and $y(0)$ should be somewhat negative if we take the origin of the spatial coordinate system to be the position of the docked neck, i.e. the free head trails behind the neck that has been pulled into place by neck linker docking. Everything is governed by the eigenvalues of the G matrix, $\lambda_\pm$. These are given by the formula

$$\lambda_{\pm} = \frac{\alpha}{2M} \pm \frac{\alpha}{2M} \sqrt{\left(1 - 4\left(\frac{M\omega}{\alpha}\right)^2\right)}$$

For a spherical approximation of the kinesin head, α is given by the Stokes formula

$$\alpha = 6\pi\eta R$$

in which the viscosity of water, η, is one centipoise and the radius, R, of the kinesin head is 3.5 nm (the head is roughly 4.5 nm × 4.5 nm × 7.5 nm). These values give

$$\alpha = 7 \times 10^{-8} \frac{gm}{s}$$

The mass of the head (340-350 aa's, or 50 kd) is

$$M = 8 \times 10^{-20} \ gm$$

Thus the Langevin relaxation time is

$$\tau_R \equiv \frac{M}{\alpha} \cong 1.14 \times 10^{-12} \ s$$

i.e. picoseconds. This is one time scale. The other time scale is the period of the elastic oscillations given by

$$\tau_O = \frac{2\pi}{\omega}$$

From above the formula for K with amino acid spacing $a = 3.5$ Å and contour length $L = 5 \ nm$ gives

$$K = \frac{3k_B T}{aL} = 6.9 \ \frac{gm}{s^2}$$

With the mass of the head given above (and neglecting the mass of the neck linker itself) this gives

$$\omega = \sqrt{\frac{K}{M}} = 0.93 \times 10^{10} \ s^{-1}$$

or a period of

$$\tau_O = 6.8 \times 10^{-10} \ s$$

This is the longer time scale. Clearly the quantity in the eigenvalue radical, $M\omega/\alpha$, is very small

$$\frac{M\omega}{\alpha} = 2\pi \frac{\tau_R}{\tau_O} = 10^{-2}$$

Thus a very good approximation to the eigenvalues in this case is given by expanding the radical and only keeping the first two terms

$$\lambda_\pm = \frac{\alpha}{2M} \pm \frac{\alpha}{2M}\left(1 - 2\left(\frac{M\omega}{\alpha}\right)^2\right)$$

The plus sign yields an eigenvalue vary close to α/M which corresponds with the picosecond time scale τ_R. However, the minus sign yields an eigenvalue of

$$\lambda_- = \frac{M\omega}{\alpha}\omega$$

This corresponds with a time scale 100 times longer than τ_O. The relaxation times for the two component process are not between the time scales τ_R and τ_O as is sometimes claimed. When the τ_R/τ_O ratio is small, as in this case, one relaxation time is indeed close to τ_R, the shorter time scale, but the other relaxation time is much longer than τ_O, the longer time scale. Even so, this long relaxation time is

only 10^{-8} s in the present case. So 2 orders of magnitude difference in intrinsic time scales leads to 4 orders of magnitude difference in relaxation times.

Once the neck linker docking is complete the free head will be moving on average at a few cm/s as previously discussed and it will be located a nm or so behind the docked neck. The longer relaxation time implies that the free head might move an additional few angstroms until its secular motion is completely damped out, not the required several nm's needed to reach the next microtubule binding site. These remarks can be made rigorous as follows.

In chapter 2 it was shown how far an *E. Coli* will drift once its flagella is turned off. The result was obtained by integrating the velocity over time to get the displacement (recall that the answer was less than an angstrom). In the present case the problem is two dimensional and $y(t)$ is the scaled displacement associated with the velocity $v(t)$. Thus we get the displacement directly from the upper component of the two component vector. We are interested in what happens just after the neck linker docking has ended. The neck has reached a position X at that moment that will remain constant up until the next kinesin head release that will happen several milliseconds later (the rate limiting step is slow phosphate release). Recall that the neck is connected to the docked neck linker that is docked on the bound kinesin head that is bound to the very massive microtubule that for all practical purposes isn't moving at all. Since even the longer of the two relaxation times is much shorter than several milliseconds, we can set $t = \infty$ in the formal solution to an extremely good approximation:

$$\begin{pmatrix} y(\infty) \\ v(\infty) \end{pmatrix} = e^{-G\infty} \begin{pmatrix} y(0) \\ v(0) \end{pmatrix} + \int_0^\infty ds \; e^{-G(\infty-s)} \begin{pmatrix} 0 \\ KX/M \end{pmatrix}$$

On the right hand side the first term vanishes because both relaxation eigenvalues of G are positive, and the second term can be integrated for constant X.

$$\int_0^\infty ds \; e^{-G(\infty-s)} = G^{-1} \; e^{-G(\infty-s)}|_0^\infty = G^{-1}$$

Thus, the displacement of the free kinesin head after neck linker docking is complete is given by

$$\begin{pmatrix} y(\infty) \\ v(\infty) \end{pmatrix} = G^{-1}\begin{pmatrix} 0 \\ KX/M \end{pmatrix} = \begin{pmatrix} \alpha/M\omega^2 & 1/\omega \\ -1/\omega & 0 \end{pmatrix}\begin{pmatrix} 0 \\ KX/M \end{pmatrix} = \begin{pmatrix} KX/M\omega \\ 0 \end{pmatrix}$$

in which G^{-1} was easily computed from G. Therefore the displacement is

$$x(\infty) = \frac{KX}{M\omega^2} = X$$

The free kinesin head has not been "thrown forward" beyond the position of the docked neck but is in fact precisely even with the docked neck, *on the average*. Notice in particular that the result is *independent* of the secondary relaxation time that has cancelled out of the final expression. In addition the distribution of positional fluctuations for the free kinesin head in the harmonic approximation is given by

$$W(x) = W_0 exp\left[-\frac{M\omega^2(X-x)^2}{2k_BT}\right]$$

in which W_0 is the normalization. This means the variance, σ is given by

$$\sigma = \sqrt{\frac{k_BT}{M\omega^2}} = \frac{1}{\sqrt{2}} \times 10^{-7} cm$$

i.e. less than a nm. The relaxation to this equilibrium positional distribution does involve the slow relaxation time, about 10 nsec. The gaussian fall-off in this distribution is already very great before the nonlinear form of the Bustamante et al. force formula is required. It would merely make the gaussian tails smaller but only after they are already very very small. Thus, even the positional fluctuations are extremely unlikely to place the free kinesin head over the next binding site. Only Brownian motion of the head can do this [1,2,3].

References

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[4]   Bustamante, C., Marko, J., Siggia, E and Smith, S., *Science* **265**, 1599-1600, 1994.