Supporting Appendix 1

Analysis of Force Production by a Bending Protofilament (PF). To identify the GTP-dependent parameters that are responsible for maximal force production by a depolymerizing microtubule (MT), we analyzed the catastrophic shrinking of the 13_0 polymer made entirely of GDP-associated tubulin. We take as a coupling device a symmetric ring whose inner diameter is larger than the outer diameter of the MT by an amount 2*w*. In this case, one can simply consider the force produced by a single PF working against a single point of contact that we call the fulcrum. This fulcrum is assumed to be at a constant but relatively large distance from the MT surface ($w >> r_o$). In this analysis, *w* will be constant, and the distance the point travels along the MT will be one dimer's length, starting from the distal end of the terminal dimer, *N*. We call this State 1. During sliding, this dimer gives the initial push against the ring and gradually becomes freed from lateral bonds, so it can bend to its equilibrium configuration. Once it is at equilibrium, the terminal dimer experiences no more bending relative to its neighbor (*N*–1), and it exerts no further influence on either the ring or on the rest of the MT. However, the (*N*–1) dimer will now start to bend. The constant value of *w* forces the ring to move parallel to the MT axis. Eventually, it will arrive at a new condition (State 2) in which the ring is at the distal end of the (*N*–1) dimer. Because the coordinates of the terminal dimers, including their bending angles θ_i (Fig. 1*A*), are insensitive to total MT length (1), the (*N*–1) dimer will assume a configuration identical to that of the first dimer before sliding. Moreover, the local shape of the PF pushing against the ring will not change, except that its terminal dimer *N* has lost its lateral interactions and is now at equilibrium. Therefore, $\theta_i^{(1)} = \theta_{i-1}^{(2)}$ for all $i = 2, ...n$, where *n* is the total number of dimers in the PF and the superscript indices refer to the states 1 and 2. The maximal work (ΔW) that is produced during the ring's sliding one dimer's length equals the energy difference between the final and initial states:

$$\Delta W = U^{(2)} - U^{(1)} = \sum_{i=1}^{N} U_i^{(2)} - \sum_{i=1}^{N} U_i^{(1)}, \qquad \textbf{[6]}$$

where U_i is the potential energy of all interactions for the *i*th dimer.

When **Eq. 6** is written in the explicit form it contains 6*N* terms, which correspond to the potential energies of 2*N* dimers. However, since $\theta_i^{(1)} = \theta_{i-1}^{(2)}$, we are left simply with terms that correspond to only two dimers: $\Delta W = U_N^{(2)} - U_1^{(1)}$. As described above, after the ring has slid to State 2, the contribution of dimer *N* is 0, thus $\Delta W = -U_1^{(1)}$, i.e., the total potential energy of the $i = 1$ (minus end) dimer. Since $\chi_1 = \theta_1 = 0$ and ${}^{\alpha,\beta}r_1 = 0$ (1),

$$U_1^{(1)} = A \cdot \left(\frac{{}^{\alpha}r_i}{r_o} \right)^2 \exp(-{}^{\alpha}r_i^2 / r_o) + A \cdot \left(\frac{{}^{\beta}r_i}{r_o} \right)^2 \exp(-{}^{\beta}r_i^2 / r_o) + B\left(\chi_i^D - \chi_{oi}^D\right)^2 / 2 = 1/2\, B(\chi_o^D)^2 \qquad \textbf{[7]}$$

where symbols α and β refer to the respective tubulin monomers, and the depth of the potential energy well for the lateral bonds is assumed to be 0.

The maximal force generated by the bending PF equals the liberated energy divided by the distance traveled (the dimer's length, *l*): $F = -U_1^{(1)} / l$. Thus, **Eq. 7** shows that the entire bending energy $g_o = 1/2\, B\,(\chi_o^D)^2$ can be used to produce mechanical work. In other words, the maximal level of the developed force (as seen on Fig. 3*C*) corresponds to the full bending energy of a stressed longitudinal bond. It should be emphasized that this analysis is applicable only if *w* is relatively large, so the dimer that becomes more distal relative to the sliding ring does not interact with the rest of the MT. When *w* is small (as in Hill's sleeve), some energy will be irreversibly lost on stretching the lateral bonds. Furthermore, the MT must have at least five dimers per PF, because in very short MTs, $\theta_i^{(1)} \neq \theta_{i-1}^{(2)}$, and some portion of the energy will be wasted.

References

1. Molodtsov, M. I., Ermakova, E. A., Shnol, E. E., Grishchuk, E. L., McIntosh, J. R. & Ataullakhanov, F. I. (February 18, 2005) *Biophys. J.*, 10.1529/biophysj.104.051789.