Supporting Text

Determination of Taxol K_D. The equilibrium dissociation constant, K_D, for botax binding to microtubules (MTs) in the presence and absence of tau was measured by the fluorimetry method of Li *et al.* [1] with minor differences.

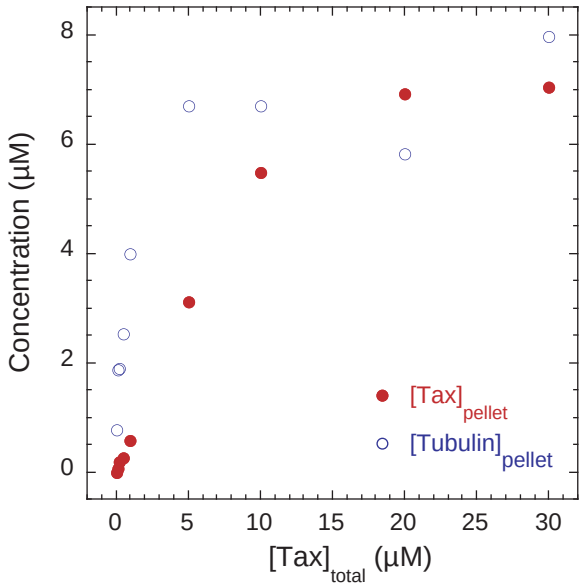
Briefly, tubulin was incubated for 20 min at 37°C to polymerize MTs. Botax was added at varying concentrations ($[Tax]_{\text{added}} = 0 - 30\ \mu$M) to a final tubulin concentration of 0.5 mg/ml, and the sample was incubated for 20 min at 37°C. For experiments testing the effect of tau, equimolar tau to tubulin was added, and the sample was equilibrated for an additional 20 minutes at 37°C.

Half of each 100-μl sample was used to determine the total botax fluorescence intensity, I_{total}, at 577 nm (excitation at 565 nm). The remaining 50 μl was centrifuged (15 minutes, $90,000 \times g$ in a Beckman A-100 airfuge), and the supernatent fluorescence, I_{sup}, was used to determine the intensity of fluorescence attributable to botax in the pellet, $I_{\text{pellet}} = I_{\text{total}} - I_{\text{sup}}$.

The resulting I_{pellet} was used as a measure of the concentration of botax in the pellet, $[Tax]_{\text{pellet}}$. The conversion factor was determined by measuring the fluorescence intensity of two standard samples: one with 500 nM botax, the other with 1 μM botax, in the presence of an excess of MTs (18.2 μM) [1]. The excess of MTs ensured that the botax in these standard samples was fully bound, like the botax in the pellet. This is important because the fluorescence intensity of botax increases 40-fold upon binding to MTs (note that a 7 nm shift in the emission wavelength, from 570 nm to 577 nm, accompanies the increase).

The tubulin concentration in the pellets, $[tubulin]_{\text{pellet}}$, was determined by fractionation via SDS/12% PAGE (tau and tubulin ran in distinct bands), and Coomassie staining relative to a tubulin standard curve on the same gel. This is different from the method of Li *et al.* who were able to use the UV absorption of tubulin to find the concentration of tubulin. Because some of our samples contained tau protein, which has tyrosine residues, the UV-absorption would not be indicative of tubulin alone.

We noted that $[tubulin]_{\text{pellet}}$ saturated at lower $[Tax]_{\text{total}}$ than $[Tax]_{\text{pellet}}$, indicating an appropriate range of $[Tax]_{\text{total}}$ was sampled. (See accompanying plot.)



Finally, botax K_D was determined by plotting the fraction of filled Taxol sites, $\langle f \rangle = [Tax]_{\text{pellet}}/[tubulin]_{\text{pellet}}$, called the fill ratio, as a function of the free Taxol concentration in solution, $[Tax]_{\text{free}}$, where $[Tax]_{\text{free}}$ was determined from $[Tax]_{\text{total}} - [Tax]_{\text{pellet}}$.

The data (shown in Fig. 3) was fit with the theoretical form for the fill ratio of a reversible binding reaction

$$\langle f \rangle \;=\; S \times \frac{[Tax]_{\rm free}/K_{\rm D}}{1+[Tax]_{\rm free}/K_{\rm D}}, \tag{S1}$$

where S, the maximum stoichiometry of botax binding to the MTs, and $K_{\rm D}$ are parameters of the fit [1–3]. In a simple one-to-one binding reaction, the value of S should equal to one.

Master Equation Method. Another method to derive Eq. **9** uses a master equation to model the binding reaction. The master equation is of the form, $\partial \mathbf{P}/\partial t = \mathcal{K}\mathbf{P}$, where $\mathbf{P}$ is a vector with dimension equal to the number of states of a binding site and whose components give the probability of being in the corresponding state. For a single binding site, there are three states, and $\mathbf{P}(t) = [e(t), u(t), b(t)]$ where $e(t)$ is the probability of the site being empty, $u(t)$ is the probability of it being filled with an unbleached Taxol, and $b(t)$ is the probability of it being filled with a bleached Taxol. The vector $\mathbf{P}$ is subject to the normalization condition $\sum_i \mathbf{P} = 1$. The rate matrix $\mathcal{K}$ corresponding to the binding reaction is given by

$$\mathcal{K} = \begin{pmatrix} -k_{\rm on}(n_u+n_b) & k_{\rm off} & k_{\rm off} \\ k_{\rm on} n_u & -k_{\rm off} & 0 \\ k_{\rm on} n_b & 0 & -k_{\rm off} \end{pmatrix}, \tag{S2}$$

where n_u and n_b are the concentrations of unbleached and bleached Taxols in solution at the binding site. We have assumed that the bleached and unbleached Taxols have the same rates.

At equilibrium, $\partial \mathbf{P}/\partial t = 0$ and the concentration of bleached Taxol bound is negligible. The equilibrium solution to the master equation is given by

$$\mathbf{P}_{\rm eq} = \left(\frac{1}{1+n_u/K_{\rm D}}, \frac{n_u/K_{\rm D}}{1+n_u/K_{\rm D}}, 0 \right). \tag{S3}$$

Notice that $u(t)$ at equilibrium is identical to the average fraction of filled sites, $\langle f \rangle$, and $e(t) = \langle \xi \rangle = 1 - \langle f \rangle =$ the average fraction of empty sites. Additionally, we define the vectors $\mathbf{P}_1 = (0, -1, 1)$ and $\mathbf{P}_2 = (-1, 1, 0)$, whose components sum to zero. These two vectors, along with $\mathbf{P}_{\rm eq}$, are linearly independent, so we can write any probability vector as

$$\mathbf{P} = \mathbf{P}_{eq} + B(t)\mathbf{P}_1 + C(t)\mathbf{P}_2, \tag{S4}$$

where the coefficient of $\mathbf{P}_{\rm eq}$ is set by the normalization condition. We assume that the initial conditions are given by $B(0) = [Tax]_{\rm free}/K_{\rm D}/(1+[Tax]_{\rm free}/K_{\rm D})$ and $C(0) = 0$. These boundary conditions ensure that, at time $t = 0$, all of the bound molecules have been bleached.

Because the diffusion of Taxol is fast, we assume that the interior of the microtubule reaches equilibrium with the outside faster than $k_{\rm off}^{-1}$. This implies that the concentration of bleached Taxol freely diffusing in the microtubule interior, $n_b(\mathbf{x})$, is due to the dissociation of bleached molecules from the microtubule and satisfies a diffusion equation subject to the boundary conditions

$$-D\frac{\partial n_b(\mathbf{x},t)}{\partial r}\bigg|_{r=R} = \frac{k_{\rm out}}{a} n_b(\mathbf{x},t)\bigg|_{r=R} - \frac{1}{a}\frac{\partial b(\mathbf{x},t)}{\partial t}. \tag{S5}$$

Here, we introduce $k_{\rm out}$ to represent the rate of Taxol passage through the pores of the microtubule wall. $k_{\rm out}$ depends on the physical characteristics of the pores and Taxol molecules and has units of (M-s)$^{-1}$. D is the diffusion coefficient, R is the inner diameter of the microtubule, a is the area of the unit cell of the microtubule lattice, and $\partial b(\mathbf{x},t)/\partial t$ is the rate that bleached molecules are entering the binding site at $\mathbf{x}$ at time t, and therefore represents the rebinding of bleached molecules.

Though it is possible to solve the bleached concentration exactly, it is productive, as in the text, to consider the limit of times longer than the time of diffusion across the lumen, R^2/D. We further assume that $k_{\rm out}$ is fast enough that bleached Taxols escape through the pores before they diffuse along the microtubule. Thus, we can neglect the bleached spot size in this limit, allowing us to specialize to the case of a homogeneous distribution of Taxol molecules along the length of the tube. In this limit, the concentration of bleached Taxols at the wall can be expressed as

$$n_b(R) \approx -\frac{4}{aR}\int_0^t dt' \exp\left[-\frac{4k_{\rm out}}{aR}(t-t')\right]\frac{\partial b(t')}{\partial t}. \tag{S6}$$

It is interesting to note that $2/aR = N_s/V$ where N_s is the number of binding sites and V is the volume of the lumen. Therefore, we see that $k_{\rm out}$ enters the Eq. **S6** multiplied by the average concentration of binding sites in the microtubule.

A similar expression can be derived for $n_u(R)$ that takes into account binding and rebinding, as well as diffusion through the pores. The difference here is that the equilibrium concentration is given by the Taxol concentration in free solution $[Tax]_{\rm free}$. Thus,

$$n_u(R) \approx [Tax]_{\rm free} - \frac{4}{aR}\int_0^t dt' \exp\left[-\frac{4k_{\rm out}}{aR}(t-t')\right]\frac{\partial u(t')}{\partial t}. \qquad \textbf{(S7)}$$

Using Eq. **S2**, the full rate matrix for the master equation can be broken into two terms: $\mathcal{K} = \mathcal{K}_0 + \mathcal{K}'$. The first term, given by

$$\mathcal{K}_0 = \begin{pmatrix} -k_{\rm on}[Tax]_{\rm free} & k_{\rm off} & k_{\rm off} \\ k_{\rm on}[Tax]_{\rm free} & -k_{\rm off} & 0 \\ 0 & 0 & -k_{\rm off} \end{pmatrix}, \qquad \textbf{(S8)}$$

where $[Tax]_{\rm free}$ is the equilibrium concentration of unbleached Taxol, represents the unbinding and rebinding of unbleached Taxol. The effect of unbinding and rebinding of the bleached Taxol is contained in the second term, given by

$$\mathcal{K}' = \begin{pmatrix} -k_{\rm on}G_b(t) - k_{\rm on}G_u(t) & 0 & 0 \\ +k_{\rm on}G_u(t) & 0 & 0 \\ +k_{\rm on}G_b(t) & 0 & 0 \end{pmatrix} \qquad \textbf{(S9)}$$

where $G_u(t) \sim \int dt' G(t-t')(-\partial_t' u)$, $G_b(t) \sim \int dt' G(t-t')(-\partial_t' b)$, and $G(t-t')$ is $\exp[-4k_{\rm out}(t-t')/(aR)]$ (Eq. **S6**).

Using this rate matrix and Eq. **S4**, we find the equations

$$\begin{aligned} \frac{\partial B(t)}{\partial t} &= -k_{\rm off}B(t) + \qquad \textbf{(S10)} \\ &\quad \left[\frac{1}{1+[Tax]_{\rm free}/K_{\rm D}} - C(t)\right] k_{\rm on} \times \\ &\quad \int dt' G(t-t')\,(-\partial_t' b) \\ \frac{\partial C(t)}{\partial t} &= -(k_{\rm off} + k_{\rm on}[Tax]_{\rm free})C(t) + \qquad \textbf{(S11)} \\ &\quad \left[\frac{1}{1+[Tax]_{\rm free}/K_{\rm D}} - C(t)\right] \times \\ &\quad k_{\rm on}\int dt' G(t-t')\,(-\partial_t' b - \partial_t' u)\,. \end{aligned}$$

Using the fact that $B(t) = b(t)$ (because only $\mathbf{P}_1$ has a component representing bleached Taxols), we will search for a solution with $C(0) = 0$. Thus, we begin with all the bound molecules being bleached. Because $C(t) \to 0$ as $t \to \infty$, we make the ansatz that $C(t) = 0$ for all t. This results in an equation for $B(t)$ given by,

$$\begin{aligned} \frac{\partial b(t)}{\partial t} &= -k_{\rm off}b(t) + \\ &\quad \left[\frac{1}{1+[Tax]_{\rm free}/K_{\rm D}}\right] k_{\rm on} \times \\ &\quad \int dt' G(t-t')\,(-\partial_t' b)\,, \qquad \textbf{(S12)} \end{aligned}$$

which is exactly Eq. **9** in the text. This demonstrates that the mean-field approach taken in the text is exact in the limit of no cooperativity.

[1] Li, Y. K., Edsall, R., Jagtap, P. G., Kingston, D. G. I. & Bane, S. (2000) *Biochemistry* **39**, 616-623.
[2] Ross, J. L. & Fygenson, D. K., (2003) *Biophys. J.* **84**, 3959-3967.
[3] Odde, D. (1998) *Eur. Biophys. J.* **27**, 514-520.