Supplementary Methods

Determining the equilibrium dissociation constant

To determine the dissociation constant of TAMRA-EDA-GTP and the exchangeable (E) site of tubulin from the observed level of labeling of microtubules, we consider chemical equilibrium between unlabeled GTP (G), fluorescently labeled GTP (F), and the E-site of tubulin (T).

GTP binds to the E-site of tubulin, forming a GTP-tubulin complex (GT). Similarly, fluorescent GTP binds to the E-site, forming a fluorescent GTP-tubulin complex (FT).

\[ G + T \rightleftharpoons GT \]

\[ F + T \rightleftharpoons FT \]

[Eq. S1, A and B]

The equilibrium dissociation constant \( K_D \) for reaction S1A is given by

\[ K_D = \frac{[G]_q [T]_q}{[GT]_q} \]

[Eq. S2]

where \( _q \) indicates equilibrium concentrations. Likewise, the equilibrium dissociation constant \( K'_{D} \) for reaction S1B is given by

\[ K'_{D} = \frac{[F]_q [T]_q}{[FT]_q} \]

[Eq. S3]

Dividing Eq. S3 by Eq. S2 and rearranging, we find

\[ \frac{[FT]_q}{[GT]_q} = \frac{K'_{D}}{K_D} \]

[Eq. S4]
By assuming that fluorescent GTP-tubulin (FT) and GTP-tubulin (GT) are equally likely to be incorporated into the microtubule, we find that the measured labeling ratio, R, is given by

\[ R = \frac{\#_{FT}}{\#_{GT}} = \frac{[FT]_{eq}}{[GT]_{eq}} \]  

[Eq. S5]

where \#FT refers to the number of labeled E-sites, and \#GT refers to the number of unlabeled sites, as described in the main text. Equations S4 and S5 will ultimately allow us to determine \( K_D/K'_D \).

To relate the initial concentrations (subscript \( i \)) to the equilibrium concentrations, we simply conserve total numbers of molecules, or

\[ [F] = [F]_{eq} + [FT]_{eq} \]  

[Eq. S6]

\[ [G] = [G]_{eq} + [GT]_{eq} \]  

[Eq. S7]

\[ [T] = [FT]_{eq} + [GT]_{eq} + [T]_{eq} \]  

[Eq. S8]

Note that Equation S8 assumes that the initial concentrations of fluorescent GTP and unlabeled GTP are much larger than the dissociation constants of 1 µM and 15 nM, respectively, so that essentially all tubulin E-sites are occupied, and \([T]_{eq}\) is 0. This condition is met experimentally. Now, we can rearrange Equation S5

\[ R[GT]_{eq} = [FT]_{eq} \]  

[Eq. S9]

Combining Eq. S8 and Eq. S9, we can eliminate \([GT]_{eq}\)

\[ [FT]_{eq} = \frac{[T]}{(1 + \frac{1}{K_D})} \]  

[Eq. S10]

or \([FT]_{eq}\)

\[ [GT]_{eq} = \frac{[T]}{(1 + R)} \]  

[Eq. S11]

To resolve Equations S10 and S11 into measurable quantities, we combine Equations S6 and S10 to solve for \([F]_{eq}\)

\[ [F]_{eq} = [F] - \frac{[T]}{(1 + \frac{1}{K_D})} \]  

[Eq. S12]

and Equations S7 with S11 to solve for \([G]_{eq}\)

\[ [G]_{eq} = [G] - \frac{[T]}{(1 + R)} \]  

[Eq. S13]

Dividing Equation S12 by S13, we arrive at

\[ \frac{[F]_{eq}}{[G]_{eq}} = \left( \frac{[F] - [T]/(1 + \frac{1}{K_D})}{[G] - [T]/(1 + R)} \right) \]  

[Eq. S14]

which is our working equation to determine \([F]_{eq}/[G]_{eq}\) from the observable quantity \( R \) and the experimental conditions which set \([F]_{eq}, [G]_{eq}\), and \([T]_{eq}\).

To calculate the ratio of dissociation constants, we then use Equations S4 and S5

\[ \#_{FT} = K_D \frac{[F]_{eq}}{[FT]_{eq}} \]

\[ \#_{GT} = K'_D \frac{[G]_{eq}}{[GT]_{eq}} \]  

[Eq. S15]

We perform a linear fit to Equation S15 to find the ratio of dissociation constants and then use literature values for \( K_D \) to finally calculate \( K'_D \).