Supplement A: The determination of $K_i$ values.

Kinetic models and fitting procedures

Control experiments provided evidence for the competitive inhibition of ATP or ADP binding to kinesin-MT by cATP or cADP. The inhibitory effects of these caged nucleotides were characterised in separate experiments to determine the value of the inhibition constants ($K_i$) for each case. The method is described here, and the $K_i$ values obtained from the analysis are presented in Table I. In the presence of inhibitor (I) the following equation describes the hyperbolic dependence of nucleotide-induced kinesin dissociation on nucleotide concentration:

$$k_{obs} = \frac{(k_{max} [N] / ([N] + K_{H} (1 + [I] / K_i)))}{k_{on}}$$

where MT is microtubule, K is kinesin and N can be either ATP or ADP, $k_{max}$ is the maximum dissociation rate constant, $K_H$ is the nucleotide concentration for half saturation, $k_{on}$ is the apparent rate constant of kinesin binding to MT, and its value is a function of the concentration of kinesin and MT. Note that the equation assumes that the re-binding of kinesin to MT was not influenced by the presence of caged nucleotides. The value of $K_i$ was determined for each case (e.g., kinesin monomer and dimer, and cATP and cADP) in separate experiments and the results are shown in Figure A1.
Figure A1: The determination of $K_i$. In flash photolysis experiments the complex formed by 0.4 µM MT and 0.8 µM kinesin monomers (a.) or kinesin dimers (b.) was dissociated in the presence of different concentrations of caged nucleotides (100-2000 µM) by releasing approximately constants (15 ± 2 µM) concentrations of ATP (filled circles) or ADP (empty circles). The figure show the measured $k_{obs}$ values. The [caged nucleotide] dependence of $k_{obs}$ was fitted with Eq. 2 in Scientist allowing all parameters to change freely to determine the value of $K_i$. For kinesin monomers $K_i$ was found to be $432 ± 30$ µM and $383 ± 40$ µM for inhibition by cATP and cADP, and for dimers $K_i$ was $477 ± 33$ µM and $394 ± 43$ µM for cATP and cADP, respectively. Solid lines were calculated by using the parameters from the fits.
Supplement B: The interpretation of intercepts from nucleotide induced kinesin-MT dissociation experiments

Kinesin-MT complexes were dissociated by ATP or ADP and the results were analysed by creating $k_{\text{on}}$ vs. nucleotide concentration plots (Fig. 1e,f). The plots show non-zero intercept values. Such intercepts could potentially be due to the effect of kinesin re-binding to MT during the course of the experiments. If this was the case, one would expect the intercept values to be dependent on the free kinesin and MT concentrations.

To test this hypothesis we have carried out the nucleotide induced kinesin-MT complex dissociation experiments by varying the protein concentrations. When we attempted to fix either the kinesin or the MT concentration the experiments proved to be very difficult due to the small amplitudes. To overcome this problem we fixed the kinesin:MT ratio at 2:1. The measured $k_{\text{obs}}$ values were analysed by using Eq. 2. The fits were carried out with $k_{\text{max}}$, $K_{\text{H}}$, and $K_i$ values fixed at those values measured in the standard experiments (Table I.) as the change in the protein concentration should not influence these parameters. The only free parameter was the intercept ($k_{\text{on}}$ in the paper).

The intercept values increased approximately linearly with increasing protein concentration (Fig. B1). This observation supported the hypothesis that the intercepts appeared due to the kinesin re-binding to MT. Using a kinetic scheme (Scheme I.) the equation describing the ADP induced dissociation of kinesin from MT can be derived with the following assumptions:
1. Step 1 and 3 are fast equilibrium steps compared to the isomerisation step 2.

2. \( K_1[D] < 1 \) and \( [K.D]/K_3 < 1 \)

3. The reaction is pseudo first order in M.K and M, i.e. \([D] \gg [M.K] \) and \([K'.D] \gg [M]\)

\[
k_{\text{obs}} = K_1 k_{c2} [D] + (k_{-2} [K.D]) / K_3
\]

The slope of the intercept vs. \([\text{kinesin.ADP}]\) plots give the second order rate constant for kinesin binding to MT \((k_{-2} / K_3)\). The derivation for ATP is more complex but has the same form where step 2 represents the net rate constant of ATP hydrolysis, \(P_i\) release and associated protein conformational changes.

According to the linear (Fig. B1) fits the second order binding constant for the single headed kinesin was \(3.2 \pm 0.3\) and \(2.7 \pm 0.4 \mu M^{-1} s^{-1}\) from ATP and ADP experiments.

Similar fits gave \(0.5 \pm 0.1\) and \(0.8 \pm 0.1 \mu M^{-1} s^{-1}\) from the ATP and ADP experiments with the double headed kinesin.

For both the single and double headed kinesin the second order binding constant was independent of the applied nucleotide (ATP or ADP). The binding of single headed kinesin to MT was approximately 4-6 times faster than the binding of the double headed kinesin.
Figure B1: The kinesin concentration dependence of the $k_{on}$. The experiments were carried out as in Figure 1. except that the kinesin and MT concentrations were varied maintaining the $2 : 1 = [\text{kinesin}] : [\text{MT}]$ ratio. The data are presented for the dissociation of single (circles) and double (squares) headed kinesins from MT by ATP (filled) or ADP (empty). Linear fits are shown as solid lines for ATP and as dashed lines for ADP induced dissociation.
Supplement C: Light scattering

The transients presented in this work are all fitted to single exponential functions and while for the most part the single exponential does give a good approximation to the data they does show significant deviations from a single exponential. We have avoided trying to fit the data to any more complex functions for a number of reasons, the main one being that the deviation were variable from experiment to experiment and in some cases from shot to shot. Below we list some of the reasons why perfect exponentials may not be expected. While we take great care to minimise these factors we cannot be sure that in every case no contribution is present form these effects.

Scattering artefacts

Light scattering is susceptible to artefacts caused by the presence of dust particles or micro air bubbles moving through the light path. We spin all solutions in a bench top centrifuge before loading in the cuvette but all particles cannot be eliminated from these viscous solutions.

Non-linear relationship between LS and concentration

Unlike fluorescence or absorbance there is no absolute requirement for a strict linear relationship between concentration of K.MT complexes and light scattering. Although an approximately linear relationship is expected the light scattering change for the first and last kinesin molecule to dissociate from a saturated microtubule may not be identical. This was considered carefully for myosin binding to actin filaments and small deviations were noted at very high and very low levels of saturation (White HD and Taylor EW, 1976, Biochemistry, 15, 5818; Finlayson B, Lymn RW and Taylor EW, 1969, Biochemistry, 8, 811).
Non-pseudo 1st order conditions (in nucleotide or protein)

To provide clean single exponentials the concentration of the reactant in excess (here the released nucleotide) should remain constant throughout the course of the observed transient. Normally a 3-fold excess is considered sufficient to give a reasonable estimate of the observed rate constant (10-15% error) but this will produce some slowing of the reaction at the low end of the concentrations used. In the case of the reaction studied here there is an additional complication of the competing reaction of K.D rebinding to the MT. Although we used a 2-fold excess of K over MT the rebinding reaction is not under true pseudo 1st order conditions and can give rise to some deviations from single exponentials.

In-homogeneous release of nucleotide from caged compounds

The flash photolysis induced release of nucleotide from the cage assumes that the concentration is uniform throughout the cuvette. While test reactions do suggest that the release of nucleotide is 95% homogeneous variations and deviations from ideality will occur.

In-homogeneity in proteins

As with any protein solution we assume that we are dealing with a single population of molecules for both kinesin and microtubules. While great care is taken with the preparations the presence of variable amounts of damaged or modified protein (misfolded, oxidised, side chain modified) cannot be totally excluded.

For all of these reasons we have not followed up more carefully the deviations from perfect single exponentials.