# Supplemental Information for

# Asymmetric friction of non-motor MAPs can lead to their directional motion in active microtubule networks

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## Supplemental Data- Figure Legends

## Extended Experimental Procedures



## Supplemental References

# Supplemental Data- Figure Legends

**Figure S1:** Biochemical characterization of proteins used in this study. Related to Figures 1,3,4,6. (A) Gel filtration profiles and associated SDS-PAGE/Coomassie stained gels for three protein constructs used in these studies. GFP-PRC1-SC on left, NuMA-tail II-GFP middle, EB1 on right. (B) Light scattering analysis of NuMA-NTD reveals it is a dimer. NuMA-NTD has a calculated molecular weight of 46 kDa. At all shown concentrations of protein examined, the molar mass of protein eluted in the peak fraction was revealed to be ~92 kDa, consistent with its eluting as a dimer. (C) Fluorescence intensity statistics for all constructs, plus GFP and dimeric GFP-PRC1-FL controls. Individual proteins were surface immobilized and visualized with TIRF microscopy (number of spots analyzed: NuMA-tail II-GFP, N=3476; GFP-PRC1-SC, N=2243; EB1-GFP, N=3193; GFP, N=4630; NuMA-bonsai, N=3598; GFP-PRC1-FL, N=1212). (D) Limited proteolysis experiments were performed using elastase and subtilisin, as described in Figure 1J, but for longer time points. Here, SDS-PAGE analysis was performed using a 20% acrylamide gel to produce better separation of low molecular weight bands.

**Figure S2:** Analysis of microtubule binding in buffer consisting of 1XBRB80 + 40mM KCl. Related to Figures 1, 3, 4. (A) Diffusion of three protein constructs (NuMA-tail II-GFP, GFP-PRC1-SC, and EB1-GFP) on single microtubules by TIRF microscopy. Kymographs showing the diffusive motion of single proteins are shown (scale bars: vertical = 5 seconds, horizontal = 2 microns). (B) Mean squared displacement is calculated for each time point, and plotted as a function of time. From the slopes, the diffusion constant for each protein can be determined, as described in the main text. (C) Comparison of diffusion constants in two buffer conditions: 1XBRB80 (used in experiments described in the text, and 1XBRB80 + 40mM KCl). (D) Measured binding lifetimes for the same three proteins.

**Figure S3:** Characterization of the optical trapping methodology. Related to Figures 2-4. (A) Uncoated beads were stuck to a coverslip in high salt (500mM KCl), and raster scanned through the trap near its focal point along both linear (X and Y) directions. (B) Examples of quadrant photo-detector signals as a function of bead position relative to the trap center for a single bead. The bead was scanned along the X dimension at various Y distances from the trap center (left plot, Y = -400 nm (cyan), Y = -200 nm (maroon), Y = 0 nm (black), Y = +200 nm (red), and Y = +400 nm (blue)). Similar data taken by scanning along the Y dimension for various X positions relative to the trap center (right). (C) To determine the viscous drag baseline for subtraction, an uncoated bead was trapped near the coverslip surface, and the stage (and therefore the buffer solution) was oscillated sinusoidally with a rate of 0.5 Hz and amplitude of 4 microns. The slope of the force-velocity relationship is well fit by a linear regression whose slope corresponds to the expected drag coefficient for a bead within 50nm of the surface. (D) The same experiment as in (C), with sparse decoration of the bead by NuMA-tail II-GFP (similar experiments with GFP-PRC1-SC and EB1-GFP were performed with nearly identical results). The data is plotted versus velocity and fit to a linear regression. (E) Comparison of the force-velocity relationship after viscous baseline subtraction for NuMA with microtubule underneath (black) and no microtubule underneath (red). The graph on bottom depicts the same no-microtubule data as on top, but the scale is enhanced to zoom in on the data; no directional bias is apparent in this control case. (F) Same as (E), but with PRC1. (G) Representative fluorescence images of polarity-marked microtubules and relative spatial distributions used for force measurements for NuMA-tail II-GFP, GFP-PRC1-SC, and EB1-GFP. Approximately equal numbers of ‘left’ and ‘right’ facing microtubules were used.

**Figure S4:** Optical trapping analysis methods and constant velocity ramp data. Related to Figures 2-4. (A) Oscillatory bead dragging experiments are performed over a range of protein/bead mixing conditions. Each time series of force data was inspected for signal (red plot) or absence of signal above baseline (black). (B) The fraction of bead/microtubule pairs exhibiting signal above baseline in each condition are plotted. (C) Calculated probability of N proteins interacting with the bead at specific probabilities of observing signal. (D) Comparison of raw time traces, with and without microtubule present. Top: NuMA-tail II-GFP decorated beads are oscillated above a microtubule. The raw data shows an oscillatory behavior, plus infrequent transient force spikes (raw data, black; processed data after spike removal, red). Bottom: A bead sparsely coated with NuMA is oscillated above the coverslip surface in a region with no microtubule present. The same transient force spikes are seen, as well as a much smaller oscillatory force signal. Blue arrows indicate specific examples of force spikes. (E) Histograms of peak force values. The magnitude and frequency of these force spikes are similar between protein/MT and protein/no-MT conditions (left: NuMA; N=15 control, N=25 with MT. right: PRC1; N=15 control, N=21 with MT). (F) Raw data time series for a PRC1-coated bead/microtubule pair using a constant velocity ramp. Force (top: raw data, black; processed data, red (positive force) and blue (negative force), microtubule position, and velocity are shown. (G) After removal of force spikes and viscous baseline force, the mean force values for individual time courses are calculated, aligned by microtubule polarity, and averaged for each velocity and protein examined (solid red and black circles; N>5 experiments per data point). Solid lines are data taken using the sinusoidal method described in the main text.

**Figure S5:** Examples of cross-linking parameters for simulations. Related to Figure 5. (A) Examples of simulation results of diffusing MT-binding domains which are dimerized with different coupling strengths. For a loosely coupled dimer, the two domains track each other, but the inter-domain distance exhibits large spreads. In contrast, a strongly coupled dimer has a linker region that provides a greater penalty for extensive separation. (B) Distributions of MT-binding domain spacing for different coupling strengths. The inter-domain spacing for each time point shown in (A) is calculated, and a histogram of these values is generated. The standard deviation of domain spacing values is presented on the plot.

**Figure S6:** Microtubule bundling by NuMA-tail II-GFP and NuMA-bonsai. Related to Figure 6. (A) SDS-PAGE analysis of NuMA-NTD and NuMA-bonsai-tail II-GFP. Red box indicates products (~30% intensity of NuMA-bonsai-tail II-GFP band) examined by LCMS-MS analysis (B) Results of mass spectrometry analysis. Bands from highlighted region in (A) (dashed red box) were excised, digested overnight with trypsin (12.5 ng/µL) at 37°C, extracted by POROS beads, and eluted by acetonitrile and acetic acid before LC-MS/MS analysis. Peptides were identified and compared with human and E.Coli protein databases. The major peptides identified were from human NuMA, as well as several peptides known to be contaminants common to MS sample preparation (e.g., keratin). (C) Microtubules are mixed with different concentrations of NuMA-tail II-GFP. At 50 nM NuMA-tail II, no microtubule bundles are detected by TIRF microscopy. At 200 nM NuMA-tail II, significant bundling is observed. (D) Microtubules are mixed with different concentrations of NuMA-bonsai. At 20 nM NuMA-bonsai, significant bundling is observed, while increased bundling can be seen at 50 nM. (E) NuMA-tail II-GFP localizes near spindle poles in spindles assembled from Xenopus egg extract. Several examples of NuMA-tail II-GFP localization when added to spindles assembled in extract at 500 nM are shown. (F) NuMA-bonsai-tail II-GFP localized at spindle poles in spindles assembled from Xenopus egg extract. Several examples of NuMA-bonsai-tail II-GFP localization when NuMA-bonsai is added to extract at 500 nM.

**Figure S7:** Analysis of NuMA-bonsai-tail II-GFP motion in perturbed bundles. Related to Figure 7. (A) “Center of mass” analysis of fluorescence intensity in microtubule overlap regions containing NuMA-bonsai-tail II-GFP. Line scans (linewidth=3, ImageJ “Plot Profile” tool) of GFP intensity are taken through the region of microtubule overlap before and after perturbation. The center of mass within the overlap region (thick lines, crosshatched) is calculated as $x_{c.o.m.} = \frac{{}_i Intensity_i * x_i}{{}_i Intensity_i}$ and plotted (dashed lines). An example of parallel (top) and antiparallel (bottom) microtubule overlap are presented. (B-C) Integrated total GFP fluorescence intensities in regions of microtubule overlap from multiple time points for parallel (B) and antiparallel (C) perturbations, corresponding to the individual traces shown in Figure 7F and 7J (coloring scheme identical to those data). (D-G) Fluorescence images, intensity linescans, and experiment schematics before and after 20 seconds of continuous perturbation of NuMA-bonsai crosslinked microtubule ‘sandwiches’. Parallel polarity-marked microtubule pairs oscillated at 5Hz (D) and 10Hz (F) show a shift in NuMA-bonsai-tail II-GFP intensity ‘center-of-mass’ in regions of microtubule overlap. GFP-intensity distribution before oscillations (top plots: orange, overlap region highlighted with hatch marks) and after oscillations (top: blue, overlap region highlighted with hatch marks) are indicated. Microtubule intensity (bottom plots, red) is higher in overlap regions and at minus-ends (due to polarity marking). Antiparallel polarity-marked microtubule pairs oscillated at 5Hz (E) and 10Hz (G) exhibit NuMA-bonsai-tail II-GFP intensity ‘center-of-mass’ positions that are similar in overlap regions before (orange) and after (blue) perturbation. Cartoon schematics are presented to illustrate bundled filament geometries. (H) Change in position of the center of mass of NuMA fluorescence after 20 seconds of continuous perturbation. NuMA moves towards the minus-ends of parallel microtubules. (Parallel, 5 Hz: N=13; Parallel, 10 Hz: N=10; Antiparallel, 5 Hz: N=12; Antiparallel, 10 Hz: N=9).

# Extended Experimental Procedures

## Protein Expression and Purification

To generate the construct PRC1-SC-GFP, amino acids 303-620 were amplified from a human-PRC1-isoform-1 clone (Pubmed accession: NP_003972) and inserted into a modified bacterial expression vector pET-DUET (Novagen), which contains a TEV protease cleavable N-terminal His-tag followed by a GFP encoding sequence, allowing for the expression of an N-terminal-GFP tagged PRC1-SC. NuMA-tail II-GFP was generated similarly: amino acids 1868-2091 were amplified from a human-NuMA clone (Pubmed accession: NP_006176.2, kindly gifted by Duane Compton) and inserted into expression vector pET-DUET between the TEV cleavage site and GFP sequence, allowing for the expression of a C-terminal-GFP tagged NuMA-tail II. EB1-GFP (amino acids 1-268) was similarly inserted into expression vector pET-DUET between the TEV cleavage site and GFP sequence.

Proteins were expressed in BL21(DE3) Rosetta (Novagen) *E. Coli* for 4 hours at 18°C after induction with 0.5mM IPTG. Cells were lysed in a French press in a buffer containing 1 mg/mL lysozyme, 50mM phosphate (pH 8.0), 300mM KCl, 10mM imidazole, 1.0% Igepal, and HALT protease inhibitor cocktail (Pierce). The lysate was clarified by centrifugation at 40,000 rpm for 45 minutes at 4°C in a Ti-70 rotor. The supernatant was incubated with Ni-NTA resin (Qiagen) for 90 minutes, washed with a buffer containing 50mM phosphate (pH 8.0), 500mM KCl, 10mM imidazole, and 0.1% Tween, then eluted with a buffer containing 50mM phosphate (pH 7.0), 150mM KCl, and 250mM imidazole. After pooling the peak fractions, the protein was incubated with TEV protease (1:30 w/w) overnight at 4°C. The next day, the protein was dialyzed against

the wash buffer (described above) for 2 hours. The protein was then passed through a NiNTA column, to which any protein with uncleaved His-tags due to incomplete TEV digestion was bound, and the eluted protein was collected and concentrated. The protein was then purified by size exclusion chromatography with a Superdex 200 column in a buffer containing 1X BRB80, 150mM KCl, and 10mM beta-mercapto-ethanol (gel filtration traces, and corresponding SDS-PAGE gel analysis for peak fractions shown in Figure S1A). The protein was finally concentrated to ~1 mg/mL in a buffer containing 30% sucrose before making aliquots and flash freezing in liquid nitrogen.

To generate NuMA-bonsai-tail II, amino acids 1-400, followed by 3 alanine residues, then amino acids 1868-2091 followed by GFP were cloned into the pGEX-6P1 vector (GE Healthcare), which contains an N-terminus GST tag followed by a PreScission cutting site. Proteins were expressed with Rosetta *E. Coli* at 18°C overnight after induction with 0.5M IPTG. Purification was performed with the following sequence of columns: (1) GST column (GE Healthcare) (buffers A: 20 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM DTT; B: A buffer plus 50 mM Glutathione reduced), (2) HiTrap S (GE Healthcare) (buffers: A: 20 mM Hepes, pH 7.4, 100 mM NaCl, 3mM DTT; B: 20 mM Hepes, pH7.4, 1M NaCl, 3 mM DTT), and (3) Superose 6 (GE Healthcare) (buffer: 20 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM DTT). The protein was collected and stored in a buffer consisting of 1X BRB80, 100 mM KCl, and 10% sucrose. A similar protocol was used to generate NuMA-NTD, which consisted of just amino acids 1-400, without the addition of the tail-II domain and GFP tag. All other expression and purification steps remained identical, except the HiTrap S column was replaced with a HiTrap Q column.

NuMA-NTD was analyzed using light scattering techniques for three different protein concentrations, as described previously (Subramanian et al., 2010). The calculated molecular weight of NuMA-NTD is 46 kDa. The protein which eluted from the Superose6 column at ~12 mL had a measured molecular weight of 92 kDa, indicating it eluted as a dimer (Figure S1B).

**Microtubule Polymerization**

Polarity marked microtubules were generated by first polymerizing bright GMPCPP seed mix, consisting of 20μM tubulin (labeled with X-rhodamine in a 1:10 labeled to unlabeled ratio), 1μM biotinylated tubulin, and 1mM GMPCPP in 1XBRB80 at 37°C for 20 minutes. Next, 15μM dimly X-rhodamine labeled tubulin (at a 1:40 labeled to unlabeled ratio), 1μM biotinylated tubulin, 15μM NEM-labeled tubulin, and 1mM GMPCPP were placed at 37°C for 1 minute, before adding 1/20 volume of the bright GMPCPP seeds. Microtubules were then allowed to polymerize at 37°C for 2 hours. Microtubules were then clarified in an ultracentrifuge (rotor TLA-120.1), and taxol was introduced to a final concentration of 20μM. To generate the non-biotinylated microtubules used in the 'sandwich' assays, the same protocol was employed, with the omission of biotinylated tubulin.

## Optical Trapping Information

*Optical trap calibration*

The trap was calibrated using standard techniques (Neuman and Block, 2004). Briefly, 1.0μm diameter microbeads were stuck to the surface of a coverslip by incubating them in the chamber with a 1M NaCl solution, which promoted strong surface binding. The stage was then scanned along each trap axis, while the voltage output from the quadrant detector was recorded. A linear regression was performed on the Voltage versus position data from the region within 200 nm of the trap center, yielding values of 1.2 +/- 0.1 V/micron in both major axes of the trap. We then trapped single uncoated beads and measured the variance of their Brownian motion, using the previously acquired position calibrations. Repeating this experiment for many beads over a range of laser powers allowed us to determine the trap stiffness as a function of power. For measurements of protein friction described in this work, we used a stiffness value of 0.04 pN/nm. For the bundled microtubule oscillation experiments, we used a stiffness value of 0.2 pN/nm.

*Trapping particle coating*

We employed 1.0μm diameter microbeads (Polysciences, catalog no. 08226-15) as trapping particles which were linked with different proteins depending on the assay. For the single molecule friction experiments, the beads were covalently linked with GFP-antibodies according to standard protocols (Polysciences Technical Data Sheet 644), allowing for the attachment to GFP-labeled NuMA-tail II, PRC1-SC, and EB1 constructs For attachment to the free microtubule in the ‘sandwich’ assay, the beads were bound with rigor kinesin, which could strongly bind microtubules without stepping or slipping

along the lattice surface. Briefly, rigor kinesin was mixed with beads in a ratio of ~$10^6$:1 in 0.1M HEPES at pH 8.0. After 20 minutes incubation, the beads were spun down and the supernatant removed, in order to eliminate unbound kinesin. The beads were re-suspended in 1XBRB80 + 0.5mM DTT + 0.5 mg/mL casein, and subsequently used in the trapping assays.

**Fluorescence Imaging Analysis**

We examined the fluorescence intensity of individual proteins, according to methods described previously (Subramanian et al., 2010). Briefly, we first flowed protein into a sample chamber to immobilize the GFP-labeled construct non-specifically to the surface. Free protein was then flushed out, and the buffer was replaced with a solution of 1X BRB80. Single images were acquired by averaging 5 frames taken at 1 second intervals using single color TIRF, with an exposure time of 200ms. After background subtraction, the intensities for each selected spot were calculated using ImageJ. NuMA-tail II-GFP, GFP-PRC1-SC, EB1-GFP, and GFP alone exhibited similar intensity values, while both the NuMA-bonsai-tail II and the known dimeric GFP-labeled version of PRC1 were shown to exhibit a mean intensity nearly twice as large, owing to the fact that each dimer contained two GFP labels (Figure S1C). We conclude that the PRC1-SC-GFP, EB1-GFP, and NuMA-tail II-GFP constructs contained a single GFP, and likely existed in a monomeric state at the low nM to sub-nM concentrations used in our assays, while the majority of the NuMA-bonsai-GFP constructs contain two GFP labels, and therefore exists as a dimer, as independently confirmed by light scattering analysis.

Diffusion of single particles was measured using the same imaging parameters, with frame rates of 1-4 images/sec. Single particle trajectories were analyzed using the Mosaic plug-in for ImageJ. Mean squared displacement and mean displacement analyses were subsequently performed using the recorded particle trajectories using home-made LabView routines.

**Optical Trapping Data Analysis**

Examination of individual time traces revealed transient force spikes. To determine whether or not such events should be included in subsequent data analysis and model fitting (that is, do they contribute useful information relating to the average force versus velocity relationship for each MAP), we examined individual traces taken with sparsely MAP-coated beads over regions in the chamber with no microtubules under the bead. Inspection of this data revealed similar force peaks, with magnitudes on the same order as those found in the case of dragging proteins across the microtubule surface (Figure S4D: top, with microtubule; bottom, without microtubule). We next quantified the magnitude and frequency of these events in both control data and data taken with a microtubule present.

We compared the frequency of such outliers in both the bead/surface and bead/microtubule dragging cases for NuMA-tail II, PRC1-SC, and EB1. Data points which were outside of 2.5 standard deviations were selected, and the maximum amplitude in each ‘spike’ was determined. After collection of these peak amplitude values for different trials and the generation of normalized histograms, we discovered that the statistical behavior of such brief force spikes is nearly indistinguishable between

conditions; that is, the source of the data spikes was predominantly independent of the MAP-microtubule interactions (Figure S4E). We attributed such large spikes to non-specific interactions between proteins on the bead and/or surface, or interactions between uncoated regions or impurities on the nominally PEG-coated coverslip and surface-blocked bead. Therefore, to obtain an accurate assessment of the force-velocity relationship for MAP/MT frictional interactions, these data spikes were subsequently removed.

In order to objectively remove these brief outliers from the raw time series, we calculated a running variance as a function of time. Large peaks in the raw data results in the appearance of large peaks in the running variance as well. Calculating the variance using a sliding window of 20 milliseconds revealed the force spikes we observed visually. After calculating the running variance for each time series, any data which was found to have produced variance values above a threshold of 2 $pN^2$ was removed from subsequent analysis. The results of this force spike removal routine are shown for two individual sample traces in Figure S4D (black = raw data, red = processed data with force spikes removed).

**Single Molecule Conditions during Force Spectroscopy**

To estimate the number of proteins interacting with the microtubule during the optical trapping assays, we performed the oscillatory bead dragging experiments using a range of bead:protein mixture ratios. We mixed 20pM of αGFP-linked beads with proteins (either NuMA-tail II –GFP, PRC1-SC-GFP, or EB1-GFP) ranging in concentration from 0.4nM to 10nM, corresponding to maximum possible bead:protein

ratios of 1:20 to 1:500. This ratio represents a potential upper limit of bead decoration; however, complete binding of all free protein is unlikely, and these values represent upper limits on surface protein density. We then performed oscillatory dragging experiments for multiple bead/microtubule pairs in each condition and counted the number of resulting force traces which exhibited signal above baseline (Figure S4A).

At low concentrations of 0.4nM protein, the data typically appeared just as control data taken in the absence of protein or microtubule, with infrequent (<10%) events exhibiting clear signals above viscous drag baseline. We conclude that the protein concentration at the bead surface was too low to make the frequent contact with the microtubule required to perform these experiments. In contrast, at the high concentration of 10nM, we observed that nearly 90% of all beads exhibited signal when dragged over microtubules, with a wide spread in peak force behavior, indicative of multiple proteins binding the microtubule simultaneously throughout the course of the data acquisition. At 2nM protein, we observed signal from approximately 30% of the bead/microtubule pairs (Figure S4B, right).

To determine the number of proteins that are interacting with the microtubule during a given experiment, we fit the fraction exhibiting signal data to a Poisson probability function of the form $P\ x\ = 1 - e^{-Ax}$, where x is the relative protein concentration and A is a fitting parameter, according to commonly employed methods (Svoboda and Block, 1994). This probability represents the likelihood that the interaction is produced by one or more protein, and that one protein at a time is sufficient to generate a detectable frictional drag event; the data were well fit by this functional form. In contrast, if two or more proteins binding at a given time were required to generate the

observed signal, the probability of measuring an event would take the functional form $P\ x\ = 1 - e^{-Ax} - Axe^{-Ax}$; this form did not fit our data well (not shown). With the knowledge that the binding between protein and microtubule is described by Poisson statistics, we calculated the distribution of interaction number as a function of percentage of beads exhibiting positive signal, given by $P\ N = k\ = \frac{\lambda^k e^{-\lambda}}{k!}$ where λ is taken from the fit statistics for each protein, and k is the possible number of interacting proteins. We conclude that at concentrations of 2nM or less, when no more than 30% of the beads exhibit signal, the likelihood of the signal arising from single protein/microtubule interactions at any given time point is at least 85% (Figure S4C).

**Determining Viscous Drag Baseline**

There are three major contributions to the measured force value during the optical trapping experiments. (1) The frictional force generated between the protein and its microtubule track and (2) the viscous drag of the bead as it is pulled through the solution throughout the experiment and (3) Infrequent high-valued transient force 'spikes' which are also seen even in the absence of microtubules. The resulting force time series data, such as that presented in the main text's Figures 2, 3 and 4, therefore consists of the sum of these contributions. To accurately assess just the frictional component of interest, the force spike outliers are removed by the automated procedure described above, and the viscous drag baseline must be properly measured and subtracted from each individual experimental trial. After this processing, the remaining force data was plotted against the calculated microtubule velocity. To generate average

force-velocity profiles for each experimental trial, the force data is 'binned' by velocity, and from all force values in a given velocity window the mean and standard deviation of force are calculated. Force-velocity profiles calculated in this way are then parsed by microtubule orientation and multiple trials for each protein are averaged, as described in the text.

To measure the viscous drag baseline, free beads were trapped just above the PEG-coated coverslip surface in the absence of both microtubules and bead-bound proteins. The sample chamber was oscillated by applying a sinusoidally varying voltage to the input of the 3D piezoelectric nano-positioning stage, using identical methods to those described in the main text. The resulting time dependent force data was replotted as a function of stage velocity, and is shown in Figure S3C. From the slope of the Force-velocity relationship, we can determine the viscous drag on the bead, β, after accounting for the correction to Faxen's Law for a bead near a hard wall, given by:

$$\beta = \frac{6\pi\eta a}{1 - \frac{9}{16}\left(\frac{a}{h}\right) + \frac{1}{8}\left(\frac{a}{h}\right)^3 - \frac{45}{256}\left(\frac{a}{h}\right)^4 - \frac{1}{16}\left(\frac{a}{h}\right)^5}$$

where a is the radius of the bead (here, 500nm), η is the viscosity of water (taken to be $8.9 * 10^{-10} \frac{pN-s}{nm^2}$ at room temperature), and h is the height of the bead center above the surface. From the fit to the data, we estimate that the bead edge sits approximately 30-50 nm above the surface.

These experiments were repeated using beads sparsely decorated with the same concentration of protein used in the microtubule friction experiments, except the beads were held above regions of the surface with no microtubules present. An

example of this data from 15 different trials with NuMA bound to the bead is shown Figure S3D (data taken with PRC1 and EB1, but no microtubule underneath, are nearly identical; data not shown). The baseline signal in the absence of protein/microtubule interactions is nearly equivalent, whether or not MAPs are bound to the bead or not.

With this baseline data in hand, we can proceed to subtract this linear relationship from all subsequent force-velocity relationships. Removing the baseline from data taken without MAP-microtubule interactions results in a null force-velocity relationship (red plots, Figure S3E and S3F; the same curves are presented in a second high resolution plot for clarity), while performing this subtraction on data taken in the presence of MAP-microtubule data results in the force-velocity relationships described in the main text (black plots, Figure S3E and S3F).

**Fitting Force-Velocity Relationships to a mathematical model**

To relate the observed frictional force to the velocity at which MAP and microtubule interact, we employ a methodology using Arrhenius-like modulations to the rate of diffusion, as described in a previous study (Bormuth et al., 2009). We will describe the key points of this derivation here.

Under zero external load, a single protein is free to hop along the microtubule lattice with a characteristic rate $k_0$. This rate is related to the diffusion constant, $D$, by $k_0 = \frac{D}{x^2}$, where x is the step size distance between equivalent and adjacent binding sites.

Under external load, the applied force will modulate the hopping rate by an Arrhenius term, according to:

$$k_+ = k_0 e^{\frac{F(0.5x+A)}{kT}} \text{ or } k_- = k_0 e^{\frac{-F(0.5x-A)}{kT}}$$

and therefore depends on the direction of application (Figure 5B, main text). Here, $kT$ is the thermal energy and $A$ represents an asymmetry distance term that varies the distance to the energy barrier which must be overcome to translocate to the adjacent site. The numerator in each exponential can thus be considered an effective 'work' applied by the externally acting force.

The velocity is then related to applied force by:

$$v = x\ k_+ - k_- \text{ , or } v = \frac{D}{x}\ e^{\frac{F(0.5x+A)}{kT}} - e^{\frac{-F(0.5x-A)}{kT}} .$$

Using this formulation, the force-velocity relationships for NuMA-tail II, PRC1-SC, and EB1 were fit using a Levenberg-Marquadt algorithm for non-linear fits, using custom-made software routines written in LabView, in order to extract the three coefficients (D, x, and A) which best fit the data according to a least-squares criteria.

**Monte Carlo Simulations**

We computationally simulated a scenario in which crosslinking microtubule-binding domains were artificially dimerized, connected by a spring-like region which acted as an effective energy penalty against excessive MT-binding domain separation, and allowed to undergo spontaneous diffusion on the microtubule lattice according to

the rates described by the simple model from the main text. All simulations were written in Python, and were run using Python 2.7 with NumPy and SciPy packages installed.

Consider two MAP domains which are defined by a single spatial parameter; their position on one of two parallel microtubules. Each domain is allowed to occupy a single site on a given microtubule, and sites are located a distance 8nm apart (as determined from optical trapping experiments, and consistent with the αβ-tubulin dimer spacing). At t = 0, both domains are located at site '0 nm'. The simulation then proceeds with a time step Δt = 0.0001s, according to the following rules:

(1) Domain 1 is allowed to step in either direction with 'hopping' probability $P_+ = k_+ * \Delta t$ or $P_- = k_- * \Delta t$. A random number P is selected with value between 0 and 1; if $P < P_+$ or $P > (1 - P_-)$, then the position of the domain is moved 8nm towards the plus or minus end respectively.

(2) This process is repeated for Domain 2, independently of the outcome from step 1.

(3) The new positions of Domain 1 and Domain 2 are compared; the lateral displacement between domains is calculated, $\Delta x$. If both domains occupy the same indexed position on each microtubule, the difference $\Delta x = 0\, nm$. If the domains have moved, the difference is non-zero, and may be either positive or negative.

(4) The effective force exerted on each domain is calculated by multiplying the displacement by an artificial spring constant: $F = k_{coupling} \Delta x$. The magnitude of the spring constant is varied so as to keep the two domains strongly (0.1 pN/nm) or loosely (0.01 pN/nm) coupled. The calculated force is then used to modify $k_+$

or $k_-$ from the relationships $k_+ = k_0 e^{\frac{F(0.5x+A)}{kT}}$ and $k_- = k_0 e^{\frac{-F(0.5x-A)}{kT}}$ respectively before the next step.

(5) The total time is incremented by Δt, and the simulation proceeds to step (1), considering the new positions and current force state of each domain. If no external perturbation is applied, the positions of the domains remain unchanged. If a non-zero oscillatory perturbation is applied, the value of the position for one microtubule is varied according to the magnitude and frequency of the perturbation.

(6) This process repeats for a given number of steps; typically a simulation will run for at least 10 seconds.

Once a simulation has finished, a linear regression of the center of mass of the dimer (found by averaging the position of the two domains) versus time is calculated. The slope corresponds to the average velocity of the artificial dimer over the length of the simulation. For a given set of conditions (i.e., dimer coupling strength, or magnitude of external perturbations), the simulation is run N=100 times, yielding an average velocity and standard deviation. We present several example time courses for multiple dimer coupling strengths (Figure S5A). Histograms of the average domain separation for these different coupling strengths are shown in Figure S5B. As we currently lack information about the mechanical properties (such as elasticity, or torsional flexibility) of the dimerization domain for both PRC1 and our NuMA-bonsai construct, we considered a range of possible stiffnesses (from 0.01 pN/nm to 0.1 pN/nm).

**Estimate of NuMA density at Spindle Pole**

In order to estimate the magnitude of frictional resistance each microtubule could experience within a dynamic structure, such as the focused aster-like structure at metaphase spindle poles, we set out to determine how many MAP microtubule binding sites might be available per microtubule in this region. We first estimate the volume of a typical spindle pole. Fluorescence images of NuMA at the 'cone-like' spindle poles suggest that it concentrates in a region that is approximately 2 microns deep and 2-3 microns in width. A cone with these dimensions has a volume of

$V = \frac{1}{3}\pi r^2 h = \frac{1}{3}\pi\ 1.5\mu m\ ^2\ 2\mu m\ \sim 5\mu m^3$. The volume of a typical mammalian cell is on the order of $1000\ \mu m^3$, suggesting that the two spindle poles occupy ~1% of the total cell volume.

To estimate the number of NuMA molecules in this region, we consider that NuMA is an abundant protein in cells, with a copy number of ~200,000 (Cleveland, 1995). Analysis of our own NuMA-bonsai-tail II-GFP fluorescence intensity suggests that NuMA is highly enriched at spindle poles, and we estimate there is a 5-fold intensity increase in fluorescence signal at poles over cytoplasm. Assuming that the localization of NuMA-bonsai at spindle poles correlates well with the distribution of endogenous full length NuMA, and considering the relative volume of pole to the entire cell, we estimate that 5% of the cell's 200,000 NuMA molecules are at poles, or 10,000 molecules in total in pole regions, resulting in a density of $1000\ NuMA/\mu m^3$. Calculations of microtubule density in spindles yield values of $50\ MTs/\mu m^3$ (Brugues et al., 2012; Heald et al.,

1997). We therefore estimate that the ratio of NuMA molecules to microtubules within spindle poles is $20\ NuMA/MT$.

# Supplemental References


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