

Correspondence

Supplemental  
Data: Monastrol  
stabilises an  
attached low-  
friction mode of  
Eg5

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Experimental Procedures

Constructs

Carboxy-terminal GST fusion proteins of rat kinesin (K430GST) and *Xenopus* Eg5 (Eg430GST) were expressed and purified by standard methods [S1]. Eg430GST was newly-constructed and fuses the amino-terminal 430 residues of Eg5.2 to the amino-terminus of GST.

Motility assays

Purified motors at 2  $\mu\text{M}$  in motility buffer (100 mM NaCl, 80 mM PIPES pH 6.9, 2 mM Mg-acetate, 5 mM DTT, 1 mM EGTA, 1.0 mM ATP, 20  $\mu\text{M}$  taxol) were supplemented with 0.05 mg ml<sup>-1</sup> casein and injected into 20  $\mu\text{l}$  flow cells so as to produce a uniform lawn of close-packed motors on the coverslip surface. Following careful rinsing with motility buffer alone, *in vitro*-assembled pig brain microtubules at 0.09  $\mu\text{M}$  were injected and their sliding motility measured from grabbed video sequences using RETRAC tracking (<http://mc11.mcri.ac.uk/retrac.html>). For the tug-o'-war assays, Eg5-GST and rat kinesin-GST motors were mixed before applying to the surface. The braking action of Eg5-GST on kinesin-GST was titratable (Fig S1) and a 1:1 molar ratio was chosen for the monastrol inhibition experiments. Monastrol was obtained from Calbiochem.

References

S1. Crevel, I.M., Lockhart, A., and Cross, R.A. (1997). Kinetic evidence for low chemical processivity in *ncd*

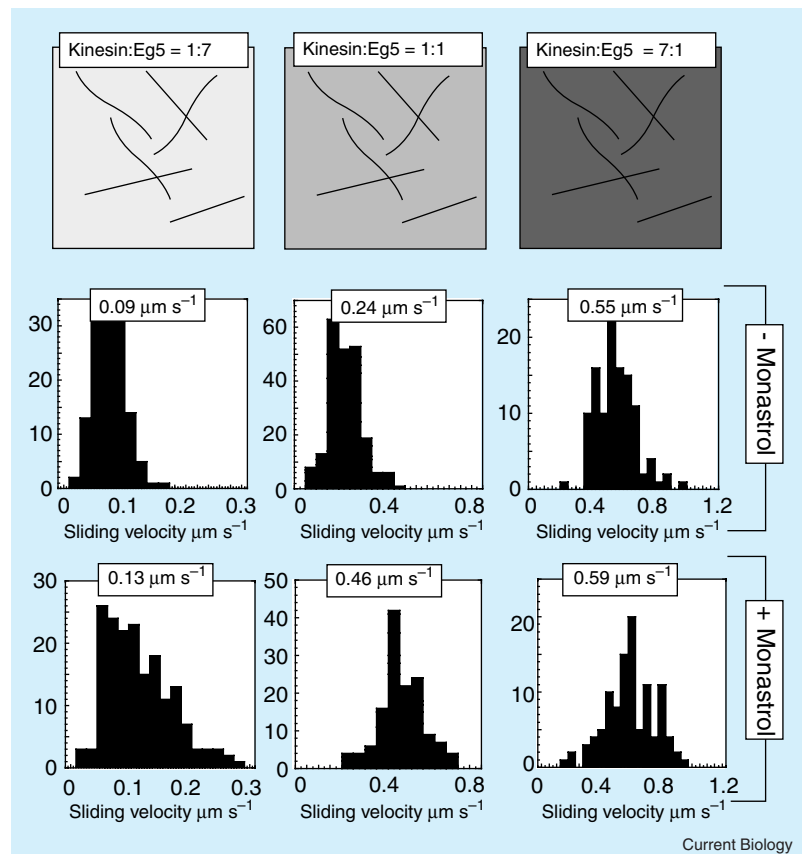


Figure S1 The effect of different ratios of Eg5-GST and rat kinesin-GST on sliding velocity in the presence and absence of monastrol.

(A) Various ratios of kinesin and Eg5 were mixed and applied to the glass surface. Kinesin : Eg5 ratio = 1:7 (left column): addition of 400  $\mu\text{M}$  of monastrol had little effect on the mean velocity ( 0.09 compared to 0.13  $\mu\text{m s}^{-1}$ ) but did cause some skewing of the velocity distribution towards slower velocities, because some microtubules moved only sporadically. Kinesin : Eg5 ratio = 1:1 (centre column): the same data as presented in Figure 1A, plotted here for comparison. Addition of 400  $\mu\text{M}$  monastrol speeds up the gliding of microtubules from 0.24 to 0.46  $\mu\text{m s}^{-1}$ . Motility remained smooth as demonstrated by the Gaussian velocity distribution. Kinesin : Eg5 ratio = 7:1 (right column): microtubule gliding velocity on this surface was very similar to that obtained on Kinesin alone. Addition of 400  $\mu\text{M}$  of monastrol produced only a small increase the mean velocity (from 0.56 to 0.59  $\mu\text{m s}^{-1}$  ) but the velocity distribution becomes more Gaussian.

and Eg5. *J. Mol. Biol.* 273, 160–170.  
S2. DeBonis, S., Simorre, J.P., Crevel, I., Lebeau, L., Skoufias, D.A., Blangy, A., Ebel, C., Gans, P., Cross, R., Hackney, D.D., et al. (2003). Interaction of the mitotic inhibitor monastrol with human kinesin Eg5. *Biochemistry* 42, 338–349.

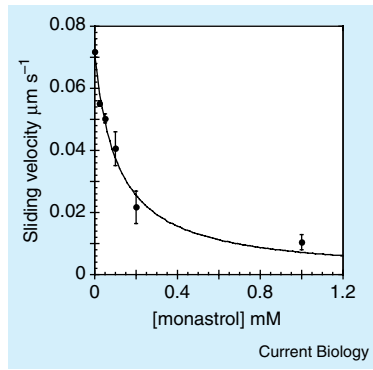


Figure S2 Titration of monastrol inhibition of *Xenopus* Eg5 sliding. Effect of increasing monastrol concentration on microtubule sliding velocity on an Eg5 surface. Conditions are the same as in Figure 1B. The fitted IC50 was 112 μM. Note that this value for *Xenopus* Eg5 is approximately 5-fold higher than the value we previously reported for human Eg5 [S2].

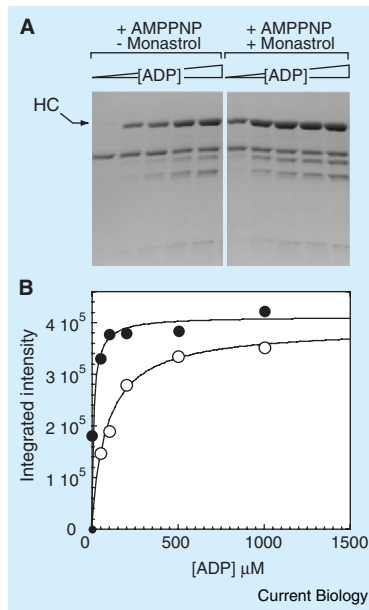


Figure S3 Conformational effects of monastrol binding.

(A) A proteolytic probe was used to sense the global conformational effects of binding of 100 μM monastrol. The experiment shown used a 5 minute digestion at 25°C of 8 μM of a single headed Eg5 construct Eg5D1-355+6his by 100 μg ml<sup>-1</sup> thermolysin in a digestion buffer comprising 80mM PIPES pH 6.9, 1mM MgCl<sub>2</sub>, 8mM CaCl<sub>2</sub>, 2mM AMPPNP, 1% DMSO, plus or minus 100 μM monastrol. Under the conditions chosen, 1mM AMPPNP induces a conformation in which the heavy chain (arrowed) is fully cleaved (first track at left). Titrating in increasing amounts of ADP in the presence of 1mM AMPPNP progressively forces a transition to the protected conformation. Addition of monastrol reinforces the effect.

(B) Densitometry of the heavy chain band gives a metric of the equilibrium constant between the two conformations. In this experiment 100 μM monastrol increases the apparent affinity of Eg5 for ADP from ~89 μM in the absence of monastrol to ~11 μM in its presence.