Structure Of A Kinesin–Tubulin Complex And Implications For Kinesin Motility

Supplementary Material

Benoît Gigant¹, Weiyi Wang², Birgit Dreier³, Qiyang Jiang², Ludovic Pecqueur¹, Andreas Plückthun³, Chenguang Wang², Marcel Knossow¹.

¹ Laboratoire d’Enzymologie et Biochimie Structurales (LEBS), Centre de Recherche de Gif, Centre National de la Recherche Scientifique, Gif sur Yvette, France.

² Institute of Protein Research, Tongji University, Shanghai, China.

³ Department of Biochemistry, University of Zurich, Zurich, Switzerland.
**Supplementary Figure 1.** Electron density map in the kinesin nucleotide binding site.

The F_{obs}-F_{calc} omit map of ADP–AlF_{4}–Mg^{2+}, contoured at the 3 σ level is presented. Kinesin and ADP carbon atoms are in green and magenta, respectively. Mg^{2+} is shown as a yellow sphere. The fluorine atoms are in cyan and aluminum is in grey. Two water molecules (red spheres) have been modeled as for Mg^{2+} to be hexacoordinated (see Methods), consistent with what has been observed in the Eg5–AMPPNP structure (Parke, C.L. et al. *J. Biol. Chem.* **285**, 5859-67 (2010)).

**Supplementary Figure 2.** Tubulin in the tubulin–kinesin complex is curved.

The tubulin structure in tubulin–kinesin (α cyan, β magenta) has been superimposed on tubulin (grey) in complex with a stathmin domain (R1, blue) that binds one tubulin heterodimer (Mignot, I. et al. *J. Biol. Chem.* **287**, 31085-94 (2012)). The region of R1 that joins its N-terminal β-hairpin to the C-terminal α helix is disordered and not presented. The r.m.s.d. of tubulin Cα positions is 0.46 Å (856 atoms superimposed out of a total of 861 atoms).
Supplementary Figure 3. The tubulin–kinesin interface after the kinesin and tubulin have been fitted separately in the electron microscopy map of kinesin-1–AMPPNP decorated microtubules. 

a. Interface with α-tubulin. b. Interface with β-tubulin.
Supplementary Figure 4. Variations of the kinesin P-loop between tubulin–kinesin and free kinesin-1.

The P-loops are compared after the central β sheets of the two kinesin structures have been superimposed. The comparison is with the undocked kinesin (pdb: 1bg2 (Kull, F.J. et al. Nature 380, 550-5 (1996))). The kinesin in tubulin–kinesin is in green and the tubulin-unbound kinesin is in yellow.
Supplementary Figure 5. Tubulin–kinesin interactions probed in an alanine scan of kinesin surface residues. **a.** The interaction of the kinesin residue Arg278 with β-tubulin. **b.** The interactions of helix H6 Glu311 residue.
Supplementary Figure 6. Comparison of the electron density maps calculated using structure factors with or without correction for anisotropy.

$2F_{\text{obs}} - F_{\text{calc}}$ maps contoured at the 1 sigma level, calculated using $F_{\text{obs}}$ corrected for anisotropy (magenta) or using uncorrected $F_{\text{obs}}$ (blue). Kinesin residues whose side-chains are significantly better defined in the corrected map are labeled.
**Supplementary Table 1** Dissociation constants of the kinesin-1 monomeric construct from tubulin and microtubules.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>tubulin</th>
<th>microtubules</th>
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<tbody>
<tr>
<td>AMPPNP</td>
<td>0.018 ±0.008 µM</td>
<td>0.045±0.012 µM</td>
</tr>
<tr>
<td>ADP</td>
<td>9.5±2.6 µM</td>
<td>2.0±0.3 µM</td>
</tr>
<tr>
<td>ADP·AlF₄⁻</td>
<td>0.22±0.02 µM</td>
<td>0.3±0.06 µM</td>
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