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

Supplementary Material

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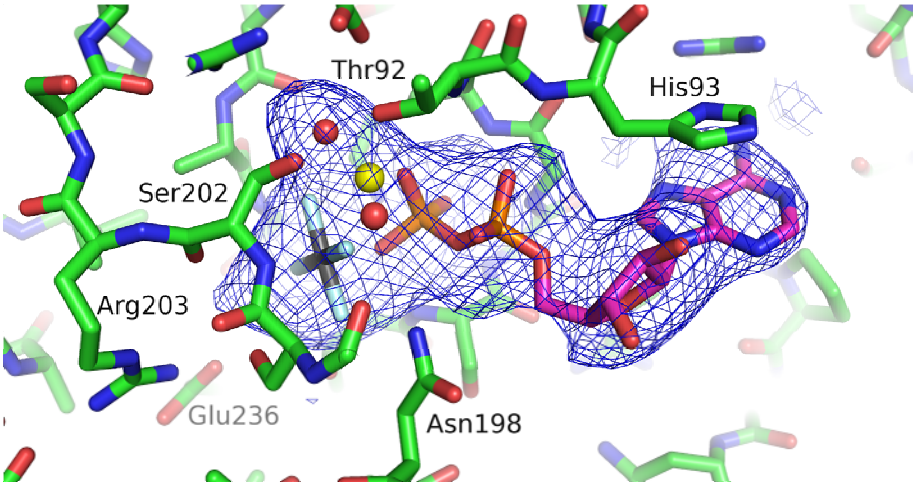
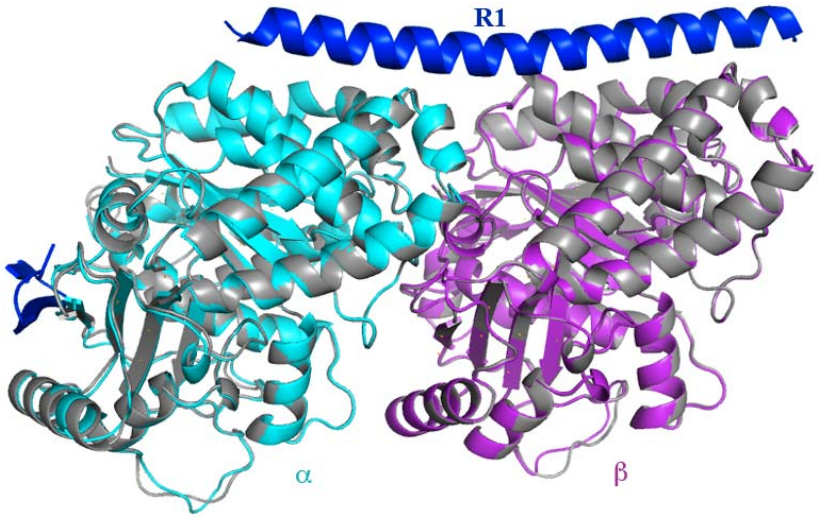
Supplementary Figure 1. Electron density map in the kinesin nucleotide binding site.



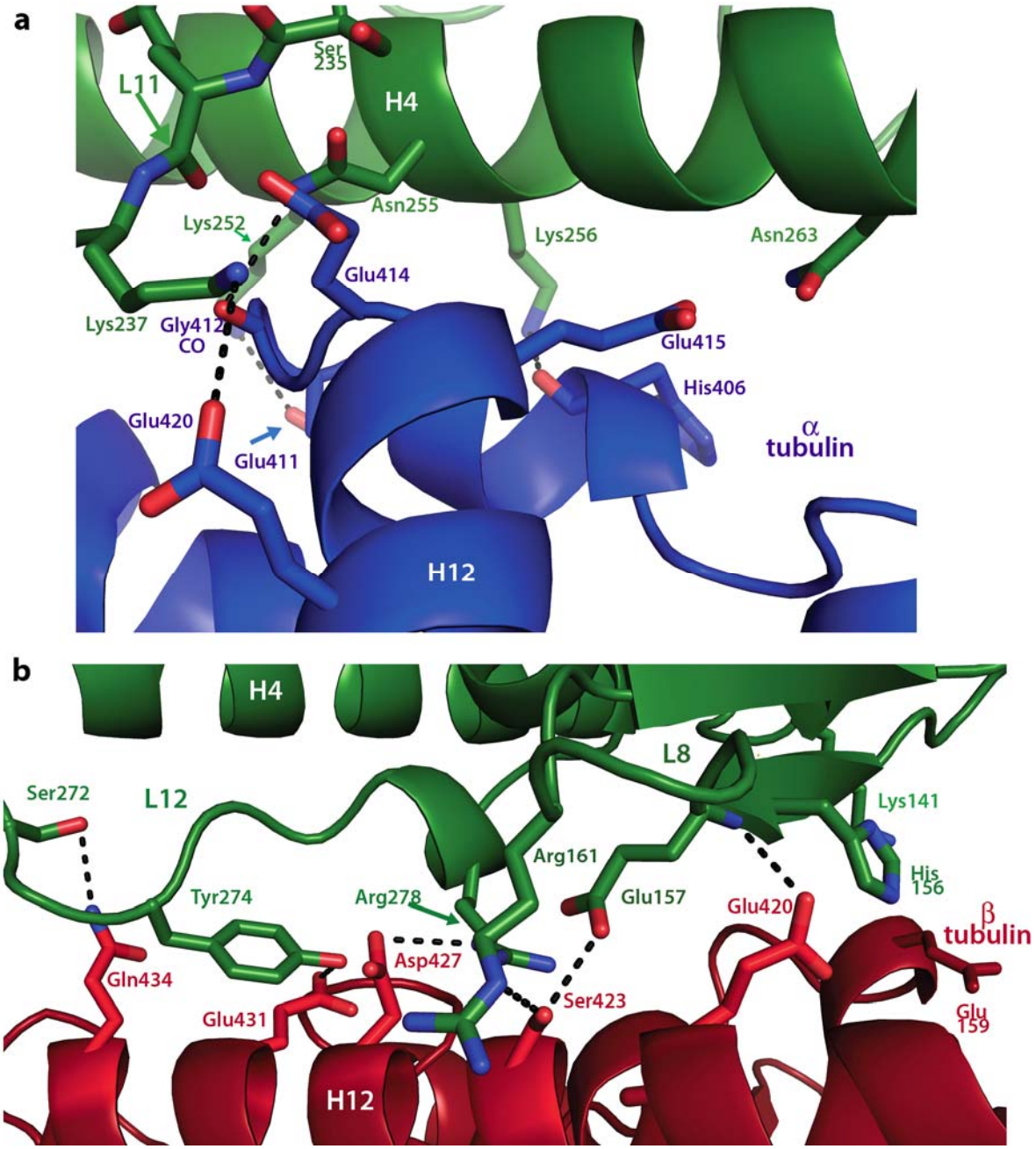
The F_{obs}-F_{calc} omit map of ADP–AlF_4^-–Mg^{2+}, contoured at the 3 σ level is presented. Kinesin and ADP carbon atoms are in green and magenta, respectively. Mg^{2+} is shown as a yellow sphere. The fluorine atoms are in cyan and aluminum is in grey. Two water molecules (red spheres) have been 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.

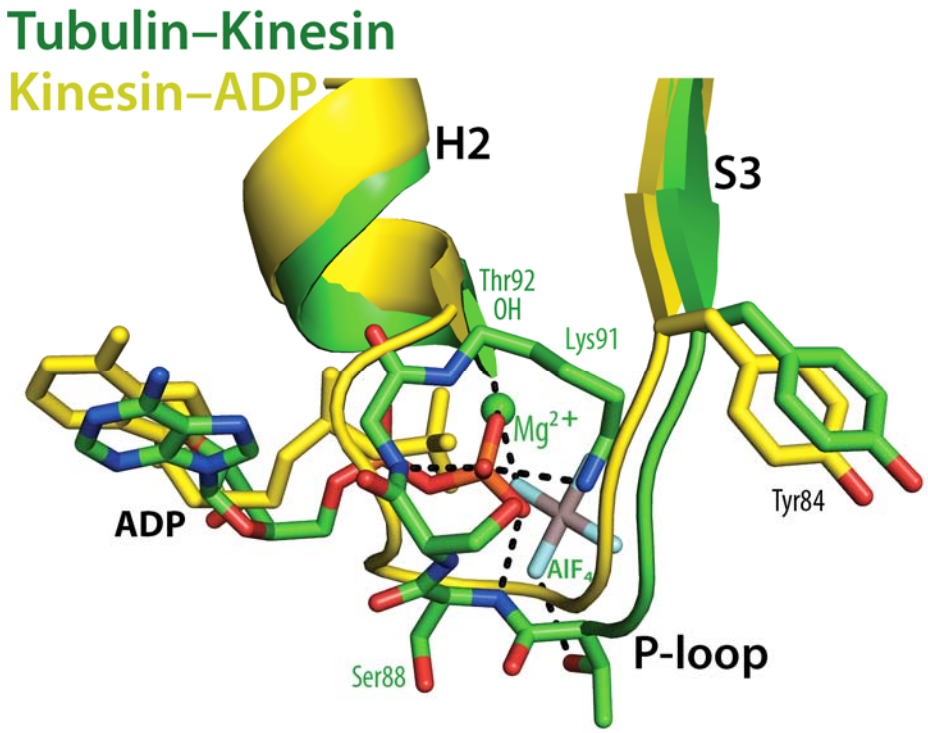


The tubulin structure in tubulin–kinesin (α cyan, β magenta) has been superimposed on tubulin (grey) in complex with a stathmin domain (R1, blue) that binds one tubulin heterodimer (Mignot, I. et al. *J. Biol. Chem.* **287**, 31085-94 (2012)). The region of R1 that joins its N-terminal β-hairpin to the 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.



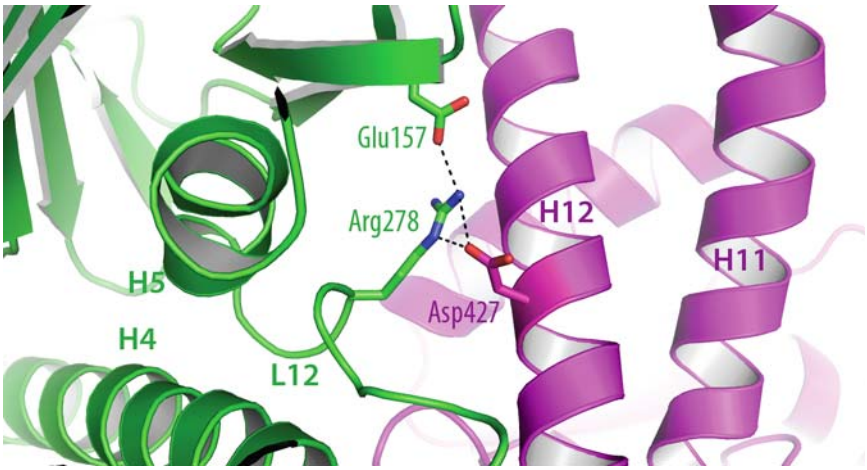
Supplementary Figure 4. Variations of the kinesin P-loop between tubulin–kinesin and free kinesin-1.



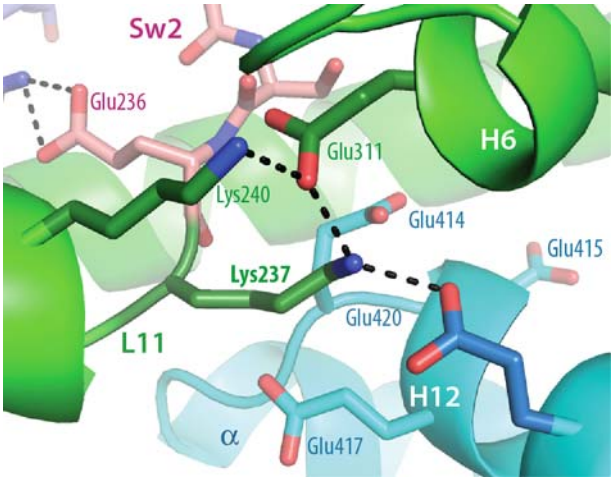
The P-loops are compared after the central β sheets of the two kinesin structures have been superimposed. The comparison is with the undocked kinesin (pdb: 1bg2 (Kull, F.J. *et al. Nature* **380**, 550-5 (1996)). The kinesin in tubulin–kinesin is in green and the tubulin-unbound kinesin is in yellow.

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.

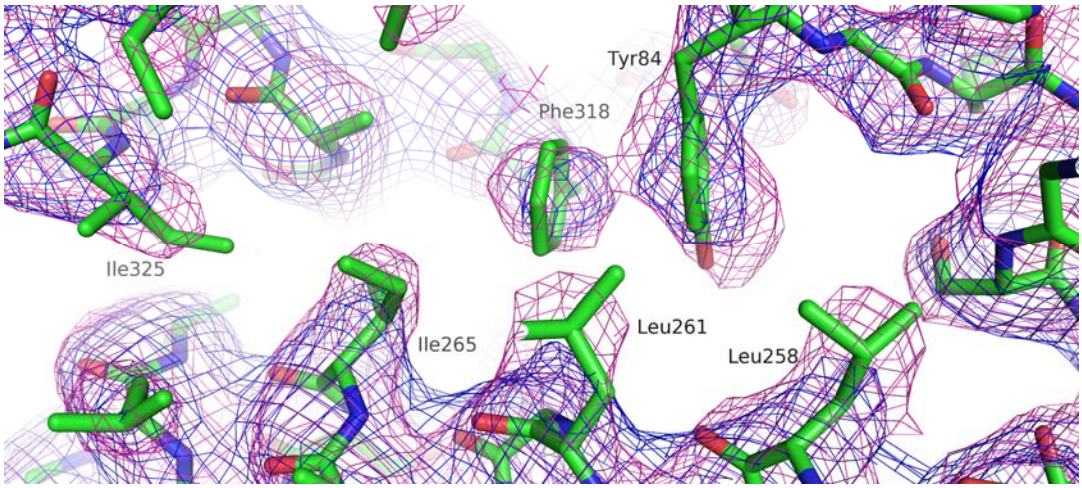
a



b



Supplementary Figure 6. Comparison of the electron density maps calculated using structure factors with or without correction for anisotropy.



$2F_{obs}$-F_{calc} maps contoured at the 1 sigma level, calculated using F_{obs} corrected for anisotropy (magenta) or using uncorrected F_{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.

Nucleotide	tubulin	microtubules
AMPPNP	0.018 ±0.008 μM	0.045±0.012 μM
ADP	9.5±2.6 μM	2.0±0.3 μM
ADP·AlF_4^-	0.22±0.02 μM	0.3±0.06 μM