P7 – Protofilament switching of Kinesin-8, investigated with Optical Tweezers

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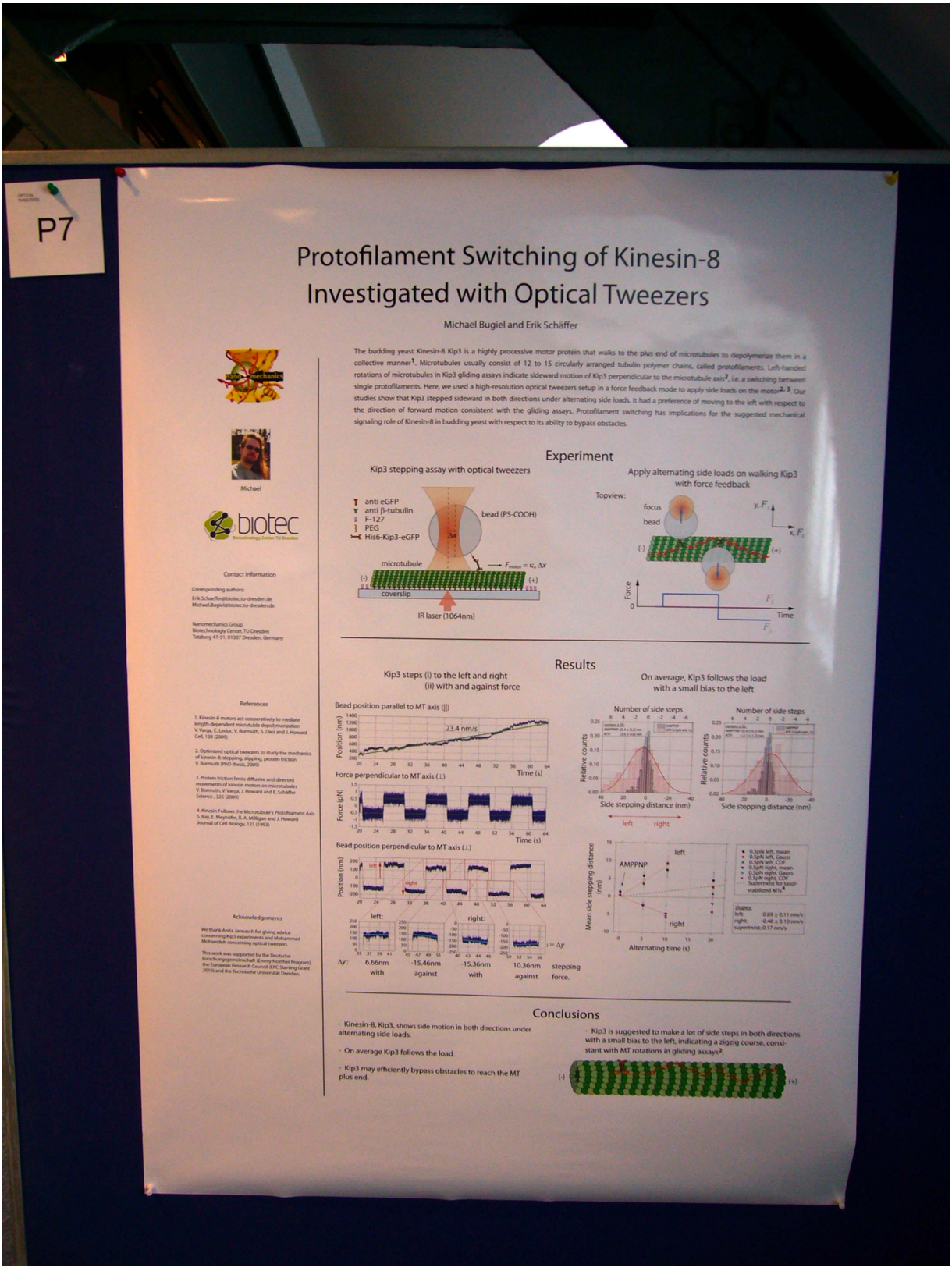
The budding yeast Kinesin-8 Kip3 is a highly processive motor protein that walks to the end of microtubules to shorten them. Microtubules usually consist of 13 circularly-arranged tubulin polymer chains, called protofilaments. Left-handed rotations of microtubules in Kip3 gliding assay indicate sideward motion of Kip3 perpendicular to the microtubule axis, i.e. a switching between single protofilaments. Here, we used a high-resolution optical tweezers setup in a force feedback mode to apply side loads on the motor. Our studies show that Kip3 stepped sideward in both directions under alternating side loads. It had a preference of moving to the left with respect to the direction of forward motion consistent with the gliding assays. Protofilament switching has implications for the suggested mechanical signalling role of Kinesin-8 in budding yeast with respect to its ability to bypass obstacles.


P7
Protofilament Switching of Kinesin-8 Investigated with Optical Tweezers
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The budding yeast Kinesin-8 Kip3 is a highly processive motor protein that walks to the plus end of microtubules to depolymerize them in a collective manner[1]. Microtubules usually consist of 12 to 15 circularly arranged tubulin polymer chains, called protofilaments. Left-handed rotations of microtubules in Kip3 gliding assays indicate sideward motion of Kip3 perpendicular to the microtubule axis[2], i.e. a switching between single protofilaments. Here, we used a high-resolution optical tweezers setup in a force feedback mode to apply side loads on the motor[2, 3]. Our studies show that Kip3 stepped sideward in both directions under alternating side loads. It had a preference of moving to the left with respect to the direction of forward motion consistent with the gliding assays. Protofilament switching has implications for the suggested mechanical signaling role of Kinesin-8 in budding yeast with respect to its ability to bypass obstacles.
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Experiment
Kip3 stepping assay with optical tweezers
anti eGFP
anti β-tubulin
F-127
PEG
His6-Kip3-eGFP
bead (PS-COOH)
microtubule
coverslip
IR laser (1064nm)
Apply alternating side loads on walking Kip3 with force feedback
Topview:
focus
bead
Force
Time
Results
Kip3 steps (i) to the left and right (ii) with and against force
Bead position parallel to MT axis (||)
23.4 nm/s
Position (nm)
Time (s)
Force perpendicular to MT axis (⊥)
Force (pN)
Bead position perpendicular to MT axis (⊥)
left:
right:
Δy :
6.66nm with
-15.46nm against
-15.36nm with
10.36nm against
stepping force.
On average, Kip3 follows the load with a small bias to the left
Number of side steps
Relative counts
Side stepping distance (nm)
left
right
AMPPNP
Mean side stepping distance (nm)
Alternating time (s)
Conclusions
- Kinesin-8, Kip3, shows side motion in both directions under alternating side loads.
- On average Kip3 follows the load.
- Kip3 may efficiently bypass obstacles to reach the MT plus end.
- Kip3 is suggested to make a lot of side steps in both directions with a small bias to the left, indicating a zigzig course, consistant with MT rotations in gliding assays[4].