RESOLVING SINGLE KINESIN MOTORS IN MOTION BY ATOMIC FORCE MICROSCOPY

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Kinesins are dimeric motor proteins involved in intracellular transport along microtubules in eukaryotic cells. Kinesin-1s are composed of two identical subunits, each consisting of a globular head of 4nm diameter and an extended α-helical stalk [1]. Single motor proteins of the Kinesin-1 family move along microtubules (MTs) in steps of 8 nm powered by the hydrolysis of ATP and can travel hundreds of nanometres before releasing from their track [2]. MTs are the largest and most rigid of the filaments that make up the cytoskeleton, the mechanical framework of the cell. The MT outer diameter is ~25 nm, while their length can reach many micrometers. MTs *in vivo* are composed of 13 protofilaments. The protofilaments run parallel to the MT axis and consist of head-to-tail joined dimers of α and β tubulin. Kinesin binds to β tubulin [3]. Recent experiments have demonstrated that kinesin moves along a line parallel to a protofilament in an asymmetric hand-over-hand mechanism, where the two heads exchange the leading position by taking non-identical alternating steps [4, 5, 6].

How exactly a single motor proceeds and how dense traffic is accommodated and regulated on the 13 narrow protofilaments of a microtubule remains unknown because the required resolution lies beyond the reach of light microscopy. A technique with which one can perform molecular dynamic imaging is atomic force microscopy (AFM). Here we have combined several recent advances in AFM methodology to visualize individual kinesin motors on MTs. We have recently succeeded to follow single Kinesin-1 dimers in their motion along microtubules with nanometre resolution by AFM in buffer. We found that both heads of one Kinesin-1 dimer are bound for the major part of the chemical cycle time to the microtubule. Furthermore, we could resolve that both heads bind to the same protofilament (fig.1), instead of straddling two, and remain on this track during processive movement.

References:

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Figures:

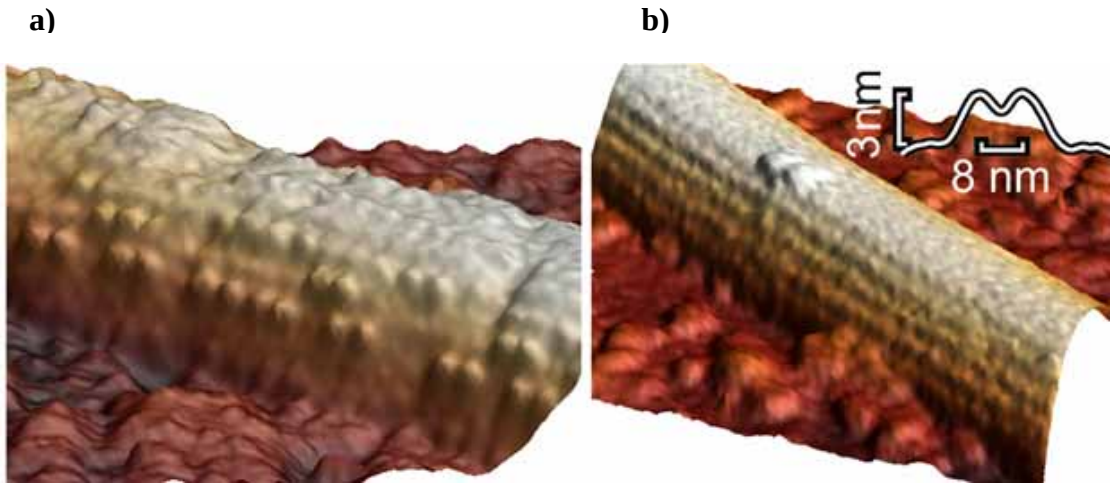

Figure 1. AFM imaging of kinesin on MTs. a) 3D-rendered image, individual motors could be clearly distinguished, heads always appeared in pairs aligned along protofilaments. **b)** 3D-rendered image, isolated motors could be seen, both heads bound on the same protofilament. Upper inset: averaged axial profile of 17 kinesin molecules, interhead spacing: 8 nm, height: 3.5 nm.