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Appendix Figures

Appendix Figure S1: Heterodimeric kinesin-2 motors were fluorescently labeled with exquisite specificity.

(A and B) FLAG-Affinity-Tag purification of the full length KLP11/20 heterodimer via the KLP20 subunit with (B) and without (A) the Halo-tag at the KLP11 subunit. Both proteins have been incubated with the fluorescently labeled peptide for labeling at the Halo-tag. Scanning of the SDS-PAGE at 642 nm showed that the only labeled protein band is the Halo-tagged KLP11 subunit (E vs. F).

(C and D) FLAG-Affinity-Tag purification of the full length KLP3A/B heterodimer via the KLP3A subunit with (D) and without (C) the SNAP-tag at the KLP3A subunit. Both proteins have been incubated with the fluorescently labeled peptide for labeling at the SNAP-tag. Scanning of the SDS-PAGE at 642 nm showed that the only labeled protein band is the SNAP-tagged KLP3A subunit (G vs. H).
Appendix Figure S2: Photobleaching analysis of the fluorescently labeled KLP3A/B and KLP11/20 motors displayed single bleaching steps in both cases.

For all constructs, the fluorescent spots for analysis were chosen by intensity and the intensity values for a 3x3 pixel window were summarized. Performing a gliding-t-value test with a corresponding threshold identified the bleaching steps.
Appendix Figure S3: Representative kymographs of the full-length KLP3A/B motor on microtubules.
Appendix Figure S4: Truncation of the run length data does not influence the results of the exponential fits (see Materials and Methods).

The algorithm for exponential fits was tested for its performance on truncated data. Columns have the same parameter $\mu$ for the exponential distribution and rows have the same truncation parameter. For truncation, all values lower than the indicated threshold are disregarded. The algorithm was used to estimate the parameter $\mu$ of the distribution. The result is given + - the 95% confidence intervals. N-numbers were chosen to be close to the lowest ones obtained for the motors described in the study.
Appendix Figure S5: Coiled coil predictions for KLP3A/B and multiple alignment of kinesin-2 tail regions.

(A) Graph depicts the predicted coiled-coil probabilities for the KLP3A (red) and the KLP3B (blue) subunits with a sliding window of 28 [1]. (B) Multiple-alignment of protein sequences shows the highly conserved proline residues in the heterodimeric kinesin-2 motors for X. laevis, D. melanogaster, H. sapiens, M. musculus, C. elegans, C. reinhardtii, and S. purpuratus. Sequence alignment was conducted as described [2]. Conserved prolines (P597 for KLP3A and P592 for KLP3B, used to create the C-terminal truncation constructs) mark the transition from predicted coiled-coil to random-coil structure of the respective subunits.
Appendix Figure S6: FLAG-Affinity-Tag purification and bleaching analysis of KLP3A/B motors.

(A) FLAG-Affinity-Tag purification of the full length KLP3A/B heterodimer via the KLP3A subunit along with its respective truncations with the SNAP-tag at the KLP3A subunit. All proteins have been incubated with the fluorescently labeled peptide for labeling at the SNAP-tag. Scanning of the SDS-PAGE at 642 nm showed that the only labeled protein band is the SNAP-tagged KLP3A subunit. (B) Photobleaching analysis of the truncated and fluorescently labeled KLP3A/B motor proteins as described in Appendix Figure S1.
Appendix Figure S7: Kymographs for single motors used for step detection in Figure 3.

Single steps (16 nm or 17 % of a pixel that corresponds to 93 nm) cannot be distinguished by eye from the kymographs (MT = microtubules, AX = axonemes).
Appendix Figure S8: Overview of the tracking procedure of a single kinesin-2 head taking steps and switching the protofilament on the microtubule.

(A) The displacements of the kinesin head are depicted as black arrows with the corresponding distances in black (all distances are given in nm). The red bars show the distance from the original head position to the current head position measured with the step tracking algorithm. (B) Resulting step detection from (A). Step sizes are calculated from the distances of plateaus in the stepping data leading to a measured step size that is smaller than the real displacement (blue numbers). This contributes to the spread of measured step sizes in the data.
Appendix Figure S9: Respective labeled filaments for the runs shown in Figure 4.

Red arrows indicate the direction, approximate length and position of the runs. Filaments are at least 3 pixels wide due to the diffraction limit. The deviations perpendicular to the filament axis in the trajectories thus correspond to only ~1/9 of the filaments’ width in the images.

Appendix Figure S10: Geometry of Biotin/Streptavidin bound microtubules to the coverslip surface.

The kinesin head together with the HaloTag fit well between the microtubule and the coverslip. With the wild-type tail, kinesin-1 lifts cargo 17 nm from the filament [3]. Considering a flexible tail, kinesin does have the space to follow a helical motion around the microtubule. For previous experiments that used microspheres or Qdots for labelling, a possibility of passage is not expected due to the size of the tags [4].
Literature