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Supporting Material

Title: Improved hidden Markov models for molecular motors. 2. Extensions and application to experimental data

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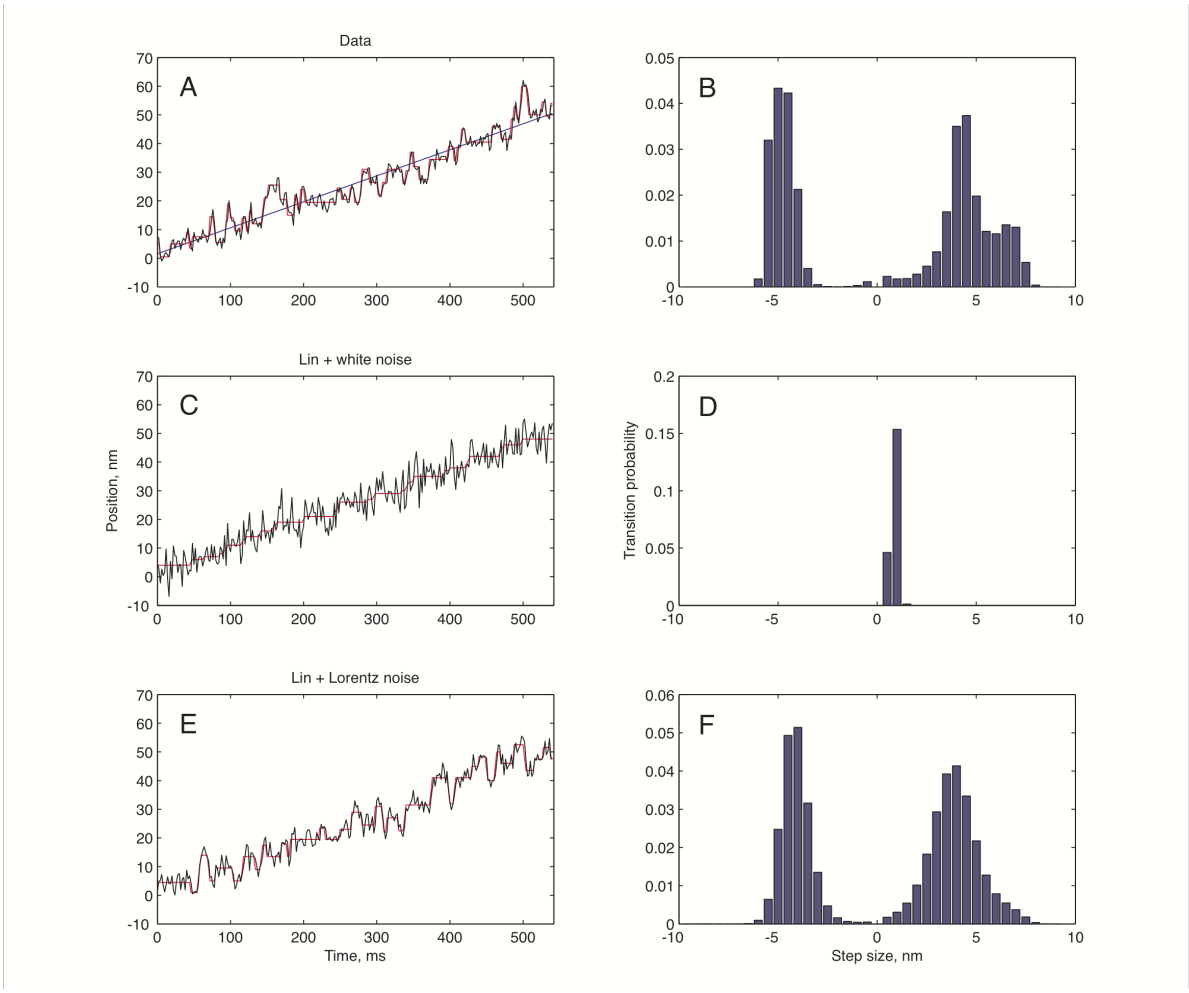

Figure S1

One-state, VSI-HMM analysis of the melanosome data of Fig. 5B is compared with the analysis of two synthetic timecourses. A, the original data are shown with the Viterbi reconstruction (red trace) and a fitted ramp (blue line). The estimated step-size distribution is shown in B. C, a synthetic timecourse was generated, consisting of a ramp with added Gaussian noise of the same standard deviation (4.1 nm) as the residuals in A. The analysis of this trace, D, yielded a model in which steps of 0.5 or 1 nm are taken at intervals of ~5 time points (10 ms), and thus makes a very good approximation to the ramp. In E, another synthetic trace was generated, in which Lorentzian noise was added to the linear ramp. The noise had the same standard deviation and the same apparent corner frequency (about 26 Hz, corresponding to a time constant of 6 ms) as seen in the power spectrum of the residual from A. Analysis of this time course by the VSI-HMM, which of course assumes underlying steps, yielded a distribution of step sizes very similar to that from the original data. We conclude that the position changes in the melanosome data could conceivably be composed of continuous Lorentzian fluctuations. However if the apparently Lorentzian fluctuations were assumed to arise from discrete steps which occur every 6-12 ms, then these elementary steps would have an approximate size of $\pm$4 nm.

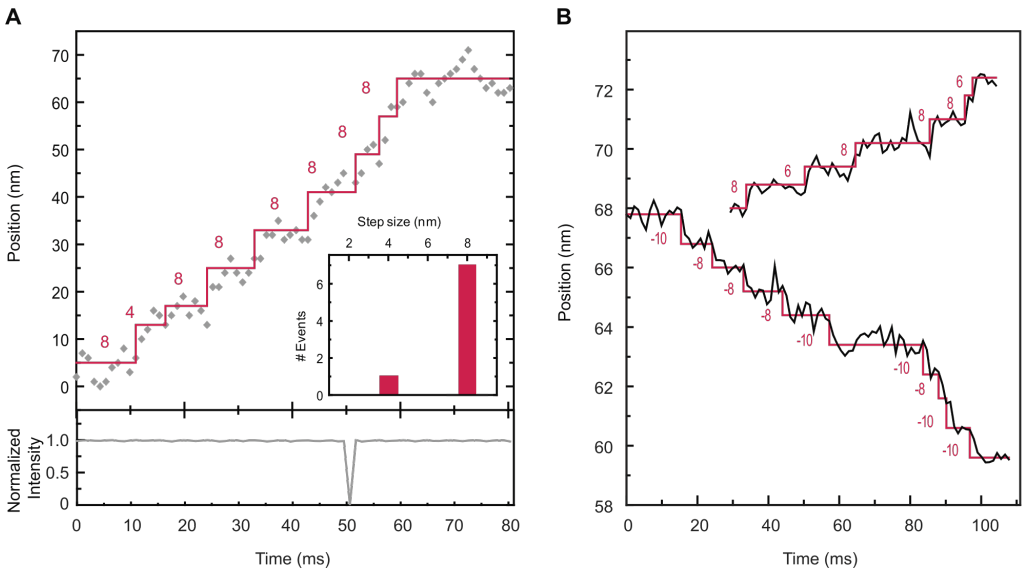

Figure S2
Additional examples of *in vivo* melanosome recordings as in Fig. 5B, reconstructed by a one-state HMM free to pick from positive or negative steps (red staircases). Here, positive steps are presumably mediated by kinesin 2 (movement away from the nucleus) and negative steps are mediated by dynein. In contrast to the data in Fig. 5B, the dominance of ~8 nm steps and unidirectional movements in these short stretches of recordings suggest activity of a single motor protein in each case. The intensity signals had only small modulations about a large mean value, with the exception of one dropped frame in (A). The data in (A) and (B) are from two independent experiments. The recordings in (B) are those of two separate melanosomes within one cell.