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Quantitative Determination of the Probability of Multiple-Motor Transport in Bead-Based Assays QIAOCHU LI, University of California Merced — With their longest dimension typically being less than 100 nm, molecular motors are significantly below the optical-resolution limit. Despite substantial advances in fluorescence-based imaging methodologies, labeling with beads remains critical for optical-trapping-based investigations of molecular motors. A key experimental challenge in bead-based assays is that the number of motors on a bead is not well defined. Particularly for single-molecule investigations, the probability of single versus multiple-motor events has not been experimentally investigated. Here, we used bead travel distance as an indicator of multiple-motor transport and determined the lower-bound probability of bead transport by two or more motors. We limited the ATP concentration to increase our detection sensitivity for multiple- versus single-kinesin transport. Surprisingly, for all but the lowest motor number examined, our measurements exceeded estimations of a previous model by $\geq$2-fold. To bridge this apparent gap between theory and experiment, we derived a closed-form expression for the probability of bead transport by multiple motors, and constrained the only free parameter in this model using our experimental measurements. Our data indicate that kinesin extends to ~57 nm during bead transport, suggesting that kinesin exploits its conformational flexibility to interact with microtubules at highly curved interfaces such as those present for vesicle transport in cells. To our knowledge, our findings provide the first experimentally constrained guide for estimating the probability of multiple-motor transport in optical trapping studies.

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