



Figure S5. **Characteristic run length and velocity data of kinesin on microtubules copolymerized with either 3RS- or 4RL-tau and stabilized with glycerol/DMSO.** Qdot-kinesin complexes were tracked on unlabeled microtubules that were formed by the addition of Alexa-488 labeled 3RS-tau or 4RL-tau (1:15 tau:tubulin ratio) to free tubulin prior to polymerization in the presence of 10% glycerol and 10% DMSO. Processive run length (plotted in  $0.5 \mu\text{m}$  bins) and velocity (plotted in  $0.1 \mu\text{m/s}$  bins) values were determined in the presence of Alexa-488 3RS-tau (A,B) or in the presence of Alexa-488 4RL-tau (C,D). The resulting processive run length histograms were fit by a single exponential decay function describing the characteristic run length  $\pm$  standard error of the fit, while the Gaussian frequency distributions of the velocity data were used to calculate the mean velocity  $\pm$  standard deviation.