

Figure S1. Accuracy of parameter estimates as a function of trajectory length. To examine the effect of trajectory length on the accuracy and variability of maximum likelihood parameter estimates, we simulated a set of 10 independent particle trajectories, each containing 10000 frames, sampled at 5 ms intervals with parameters  $D_1 = 0.1 (\mu m)^2/s$ ,  $D_2 = 0.01 (\mu m)^2/s$ , and  $p_{12} = p_{21} = 0.1$ . The full length trajectories were truncated at specific lengths indicated on the x-axis, and the maximum likelihood parameter estimates were calculated from the truncated trajectories. The relative error (top panel) and dispersion (bottom panel) in the maximum likelihood parameter estimates are plotted as a function of the truncation length.