

Figure S2. Pure Brownian diffusion analyzed with a two-state HMM. 10 independent trajectories with 1000 steps each were simulated with a diffusion coefficient of  $0.01 \, (\mu m)^2/s$ , and fitted to a 2-state HMM. As discussed above, the 2-state HMM collapses to a 1-state model in one of two possible ways: Either the two diffusion coefficients converge to the same value (A. and B.), or one of the states is inaccessible because of a very small transition probability leading into it (C. and D.)  $D_1$  and  $D_2$  are in units of  $(\mu m)^2/s$ .