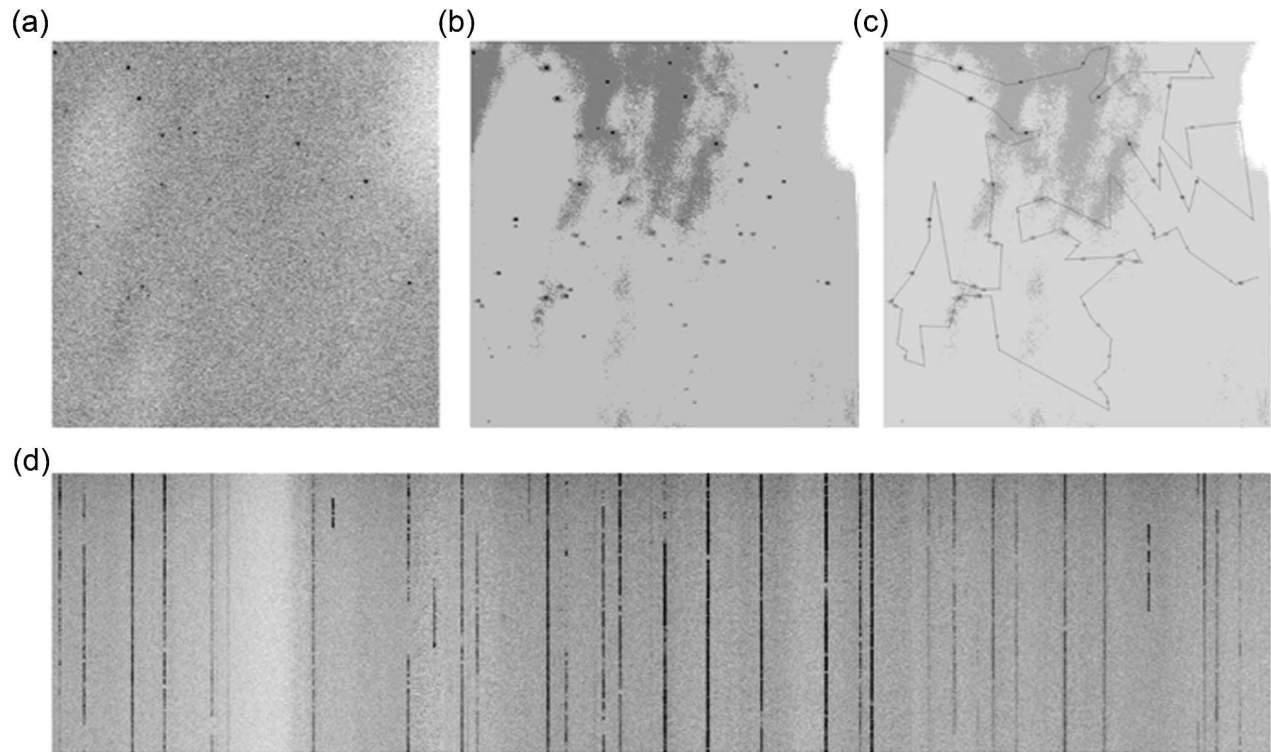


Supplemental Figure 1.



Supplemental Figure 1. Method to determine rate of dissociation of QD-MLCK from surface-attached SMM relating to Figures 6 and 7. (a) Single representative frame inverted from an image sequence collected at 11.4 frames s⁻¹. Dark spots are individual QDs. (b) Representative z-stack average image (ImageJ Version 1.47 64 bit). (c) To create a kymograph for every visible QD (2008 31 10 Kymograph macro for ImageJ by J. Rietdorf and A. Seitz (<http://www.embl.de/eamnet/html/kymograph.html>) we drew a region of interest (line) through all the QDs on the z stack. (d) Kymographs defined by the region of interest shown in (c). Lifetimes of true dissociation events using the criteria described in Methods was obtained by counting pixels (above about 160 grey scale intensity) using Photoshop CS5 software and sorted and collated in Excel 2011 (Version 14.2.4) then plotted and fit in Origin 8.6 (Home version) running in Windows 7 Enterprise, under Parallels Desktop 7.0. We measured the intensity of background

pixels and computed the 1.5 times the interquartile distance (using JMP) to determine the lowest intensity we could identify as an outlier of the background pixel intensities. Values of 167 or higher on a scale of 264 were considered above background. The 8th and 10th kymographs from the left are examples of bona fide events because any gaps in these kymographs were <7 frames long and were therefore considered to be blinks and not dissociation events. The 2nd kymograph was not used because rebinding occurred at the same location after an initial dissociation. The 3rd kymograph was not used because the QD remained bound for the duration of the movie.