Protocol 6: Experimental observation of the protein tracking with depolymerizing microtubule ends.

6.1. Introduce 30-50 µL of fluorescent protein (0.1-20 nM) into the chamber at 10 µL/min. If protein sticking to the coverslip is evident, supplement the motility buffer with 4-8 mg/mL BSA. Alexa488-Dam1 tip-tracking additionally requires 10 mM DTT or 0.5-1% βME.

6.2. Limit the illumination field using a microscope field diaphragm to avoid the unnecessary bleaching and disassembling of the microtubules.

6.3. Start video acquisition using GFP filter cube, then switch to Rhodamine filter cube without interrupting the image recording. The red segments at the microtubule ends should be clearly visible; they will begin to fade and disintegrate quickly.

6.4. Continue to illuminate until the caps almost disappear (usually for 10-20 sec but this time will be longer for the caps grown with a lower Rhodamine labeling ratio), and switch back to the GFP channel to record protein tracking with microtubule disassembly.

6.5. Analyze the resulting sequences by constructing kymographs, i.e. two-dimensional images that show fluorescence intensity along microtubule axis for various times during observation) using MetaMorph, freely available ImageJ or other image processing software.

Note 1: Acquisition rate should be adjusted depending on the timing of the observed events. The recommended rate is 2-3 frames per second (fps) for the slow-moving, ring-sized Dam1 complexes but acquisition time for single molecules should be > 20 fps.

Note 2: A highly sensitive EMCCD, e.g. ANDOR iXon3, is required for the fast recording of tip-tracking events with depolymerizing microtubules. The recommended settings for Andor iXon3 camera are: gain 5x, EM gain 200, 1MHz readout speed, 16-bit sensor mode, 80ms exposure time.

Note 3: Using TIRF microscopy will improve signal-to-noise ratio, however, shorter microtubules should be used, such that the fluorescent stabilizing caps remain within the reach of evanescent field.