Microtubule Binding Assay
Protocol

1) Thaw concentrated (~20-40 uM) recombinant Dam1 complex on ice and dilute 1/100 in 50 mM sodium phosphate, 350 mM NaCl.
2) Using wide-bore tips add 2.5 nM taxol stabilized microtubules to 1.7 mL siliconized tubes (company) containing 1x BRB80 and 10 uM taxol. (Measure a minimum of 10 ul with wide-bore tips in order to ensure accuracy.)
3) Add protein buffer so that the final volume is the same for each concentration after adding protein.
4) Add binding protein last to a maximum of 8 different concentrations.

5) After 10 minutes at RT fix samples with 2% glutaraldehyde (in BRB80) for 15 minutes. Dilution with glutaraldeyde should be 4:1. (Volume for assays were 250ul, so I fixed with 750 ul glutaraldehyde/PBS)
6) Prepare TLS55 centrifuge tubes (Beckmann number 347356) by placing a custom made spacer (Ellard Instrumentation Ltd., Monroe, WA) in the bottom of each tube.
7) Coat round 5 mm diameter glass coverslips (Bellco Glass, Inc, Vineland, NJ) in polylysine.* Mark an asymmetric 3 on one side of each coverslip and place (3 facing down) one coverslip on each spacer. Avoid scratching the coverslip by handling on an edge with small forceps.
8) Add 1 mL of 1:1 (v/v) 15% glycerol:PBS while pipette tip is pressed on coverslip (to keep it from moving).
9) Load desired volume of your sample, if larger than 250 ul, reduce cushion volume. (optimal amounts to spin down need to be worked out empirically per experiment) Spin down microtubules at 45,000 rpm (135,000 g) for 10 mins at 25◦C.
10) After spin, aspirate off the volume of your sample, then the cushion. Make sure to remove your sample before reaching the coverslip otherwise you will pick up reaction products that were not spun down.
11) Invert coverslips onto 5 µL citifluor and squash using Kimwipes to cover slide. Paint round coverslips with nail varnish. Remove the 3 with ethanol, and wash coverslip with dH20. You can get 2 coverslips on one slide.

*Make up 1mg/ml stock of polylysine. Wash coverslips with nitric acid, and wash with copious amount of water. Incubate coverslips overnight in polylysine at RT. (You can coat about 100 coverslips) Wash again with copious amounts of water. Separate individual coverslips onto kimwipes to dry. Do not use coverslips that stick together.