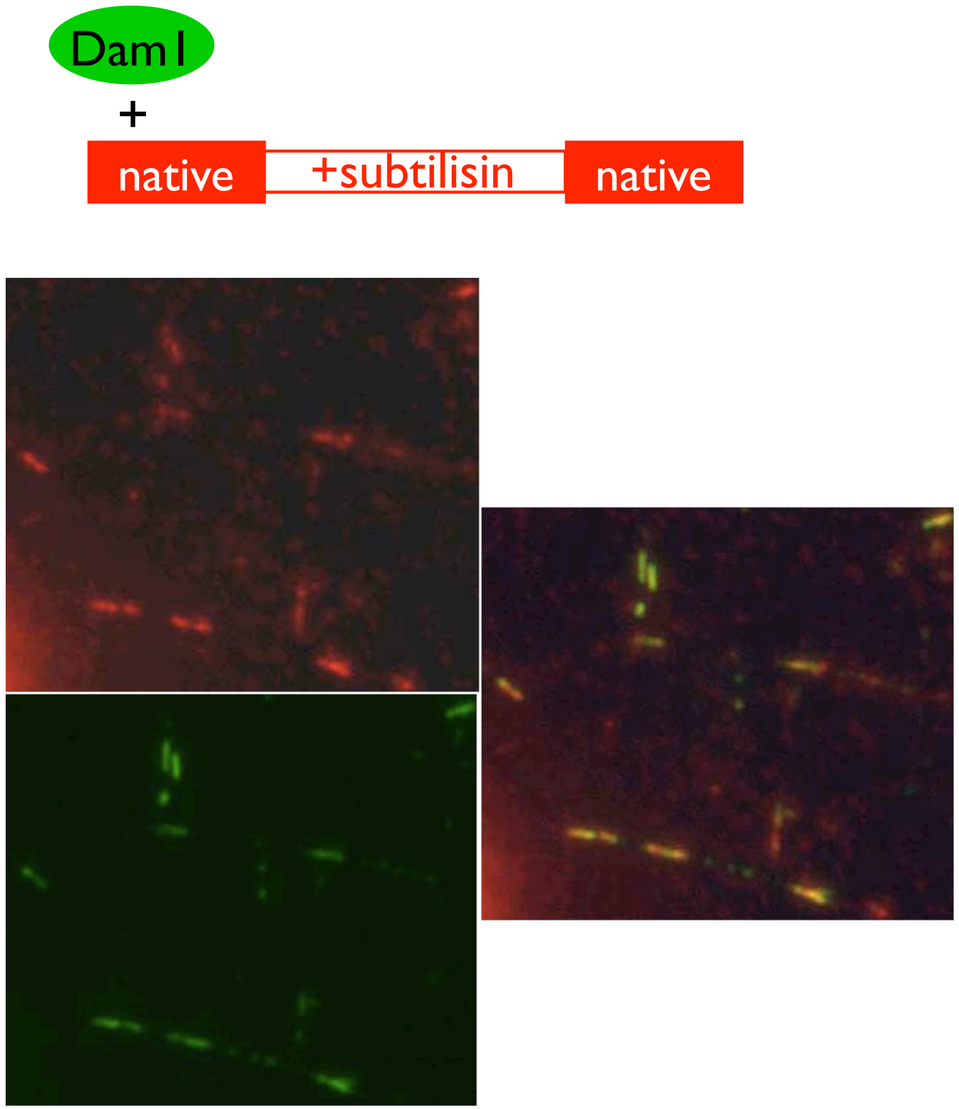
a)




b)

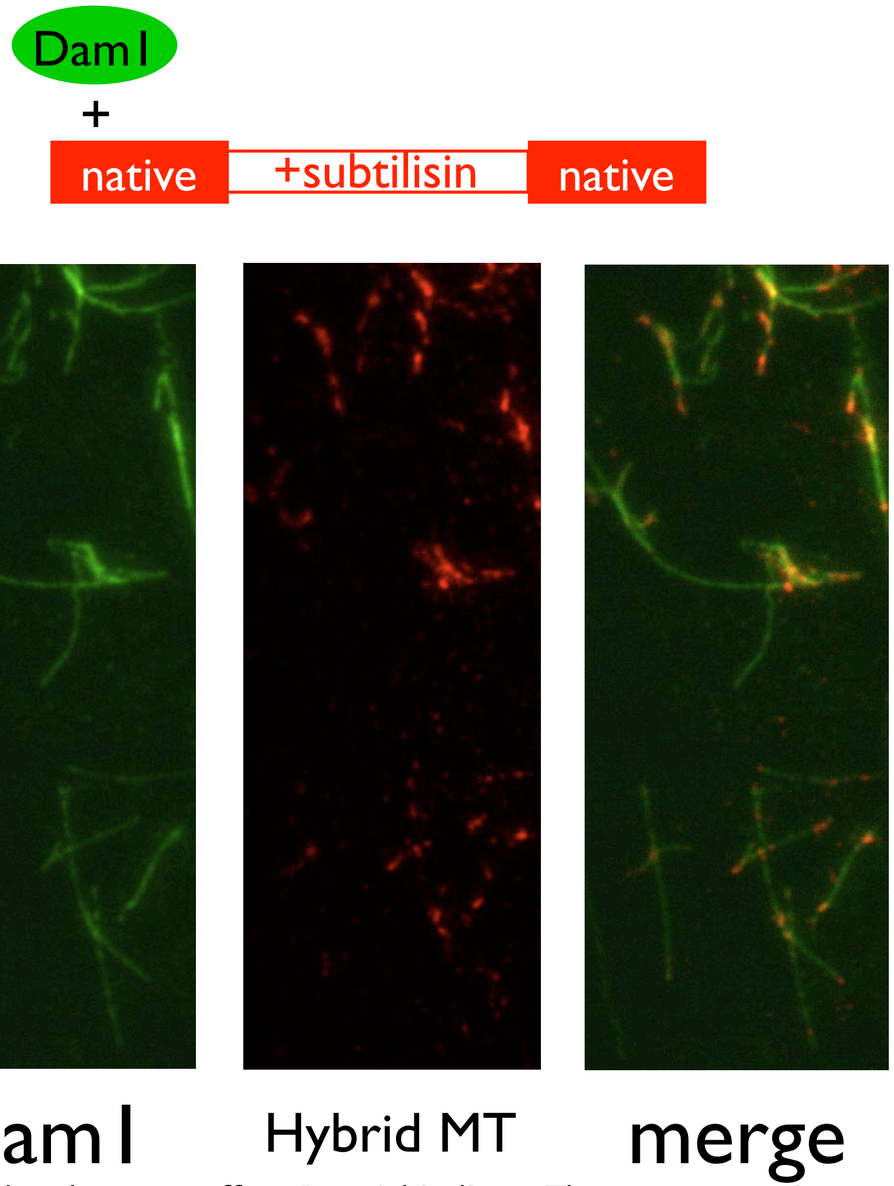



**Figure S1** – a) Control experiment to show that the rhodamine dye used to visualize microtubules does not affect Dam1 binding. The same experimental setup is used as in Figure 1, creating hybrid microtubules with sections containing and missing the E-hook of tubulin. However, the microtubule lattice labeled with rhodamine was that made of native tubulin, instead of the subtilisin-treated tubulin that is shown in Figure 1. The same preference for the native lattice is seen, supporting the conclusion that the tubulin E-hooks contribute in a significant manner to affinity for the Dam1 complex, as they are responsible for the observed difference in binding affinities (not rhodamine). b) At high concentrations, the Dam1 complex is able to saturate the microtubule lattice completely, regardless of whether or not it contains tubulin E-hooks. In these images Dam1 is added in a 2:1 Dam1 to tubulin molar ratio. Thus, although we see a clear preference of the Dam1 complex for the native tubulin lattice, this preference is obscured at saturating concentrations of the complex. Our results explain the apparent contradiction in the literature (Miranda et al., 2007; Westermann et al., 2005) as due to different concentrations of the Dam1 complex with respect to tubulin.

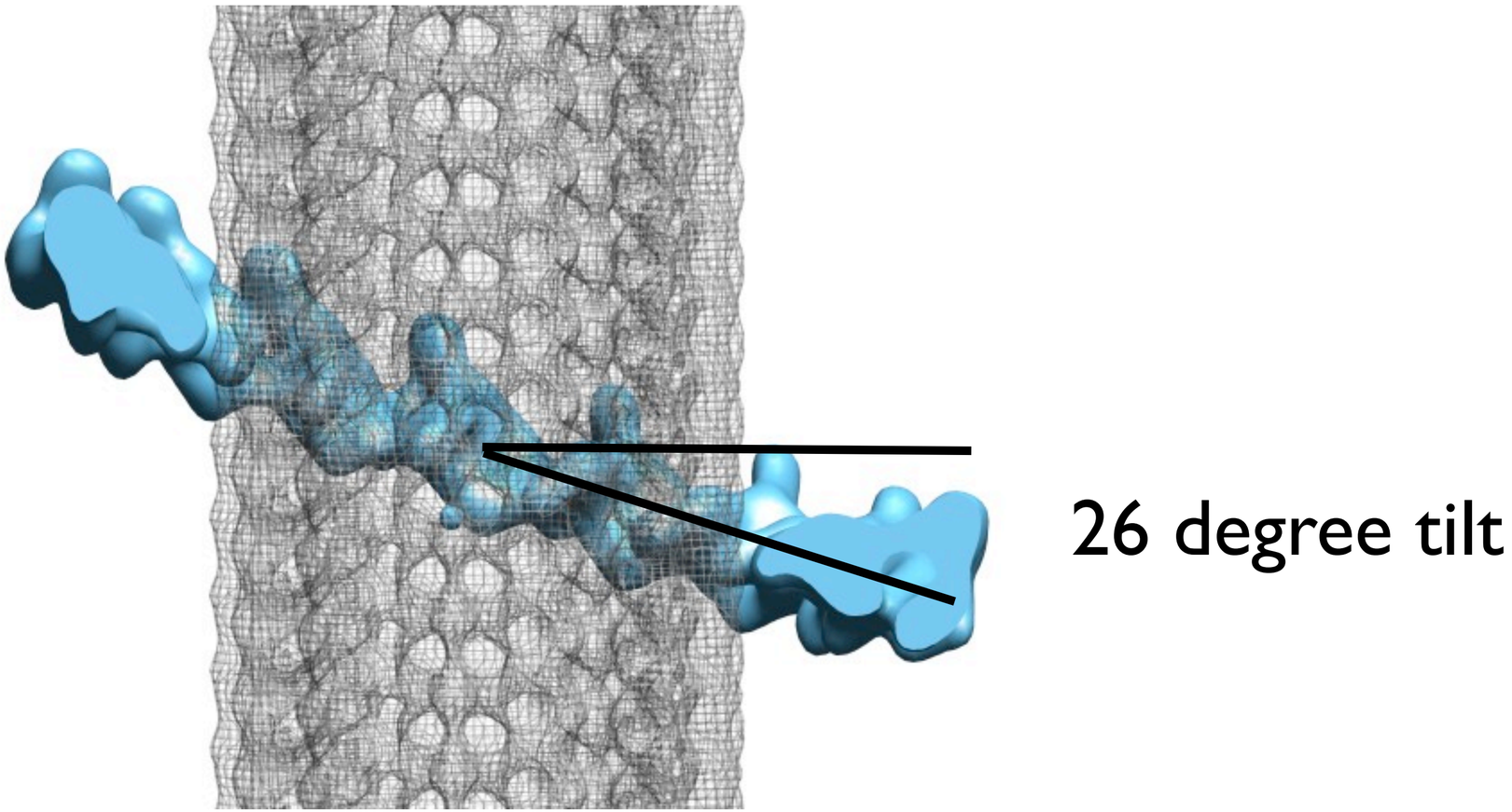



**Figure S2** – Model showing the steric clash that occurs between the Dam1 ring and the microtubule lattice when the ring is tilted to the outer range of the tilts observed experimentally (see histogram in Figure 4c). The protruding densities collide with the outer wall of the microtubule.

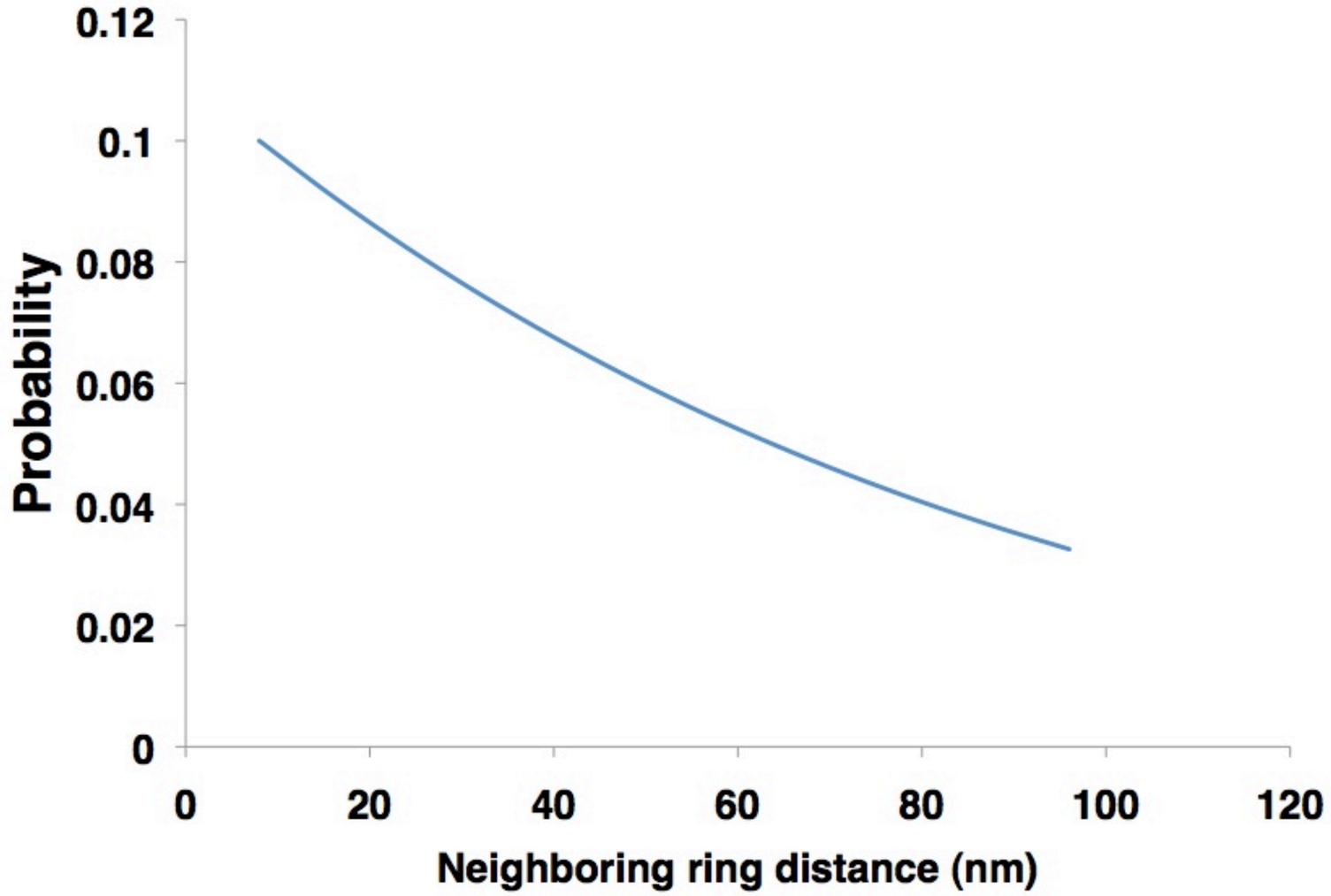
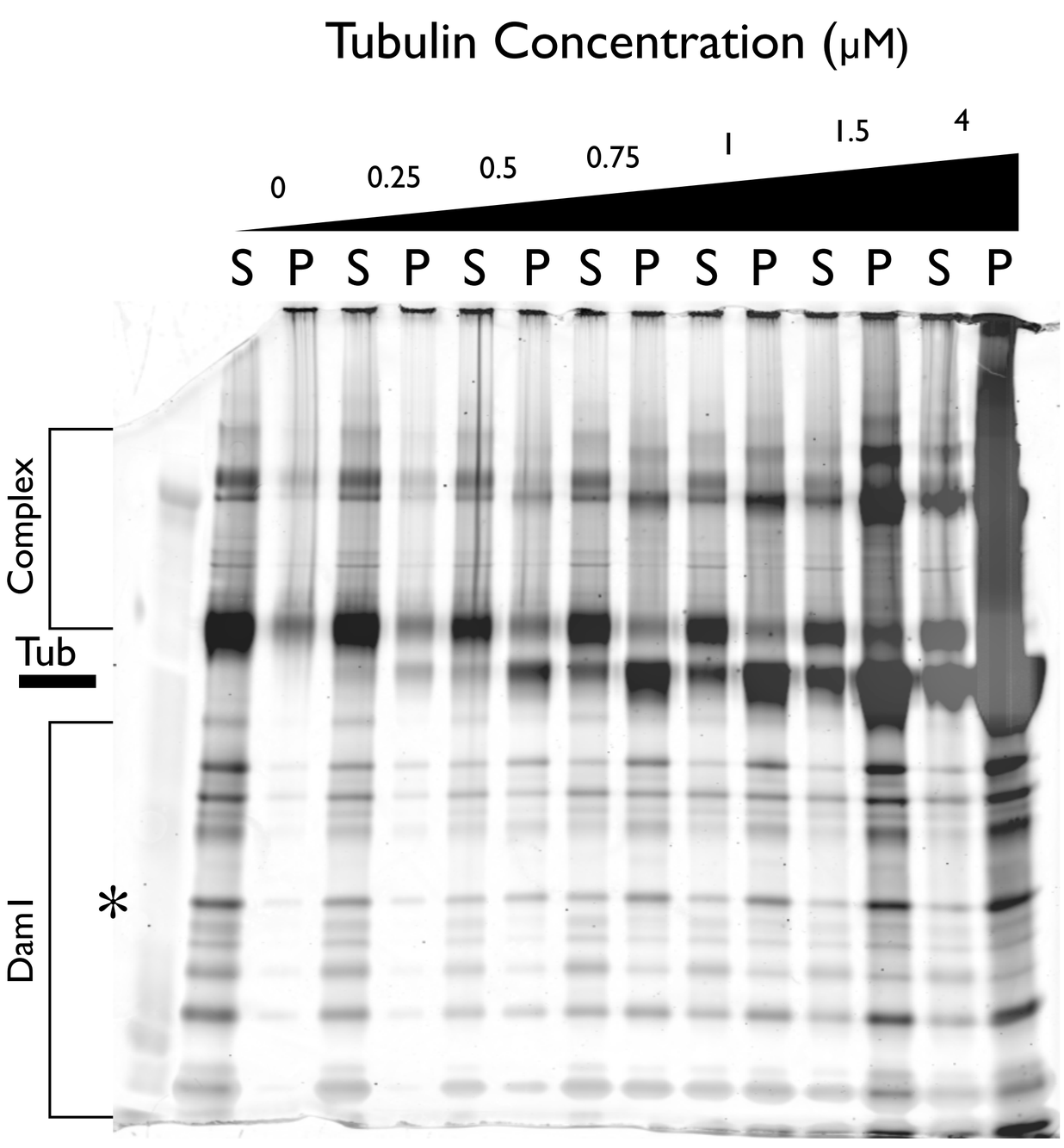



**Figure S3** – Theoretical distance distribution for neighboring rings. This curve was calculated by using a "balls in a box" model, where each ring sits in a discrete location and only one ring can occupy a box at a time. Plot calculated with 10 balls and 100 boxes. X-Axis is the number of boxes of distance between neighbors and the Y-Axis is the probability of that arrangement. For our case, the box is 8 nm, the repeating unit size on a microtubule.



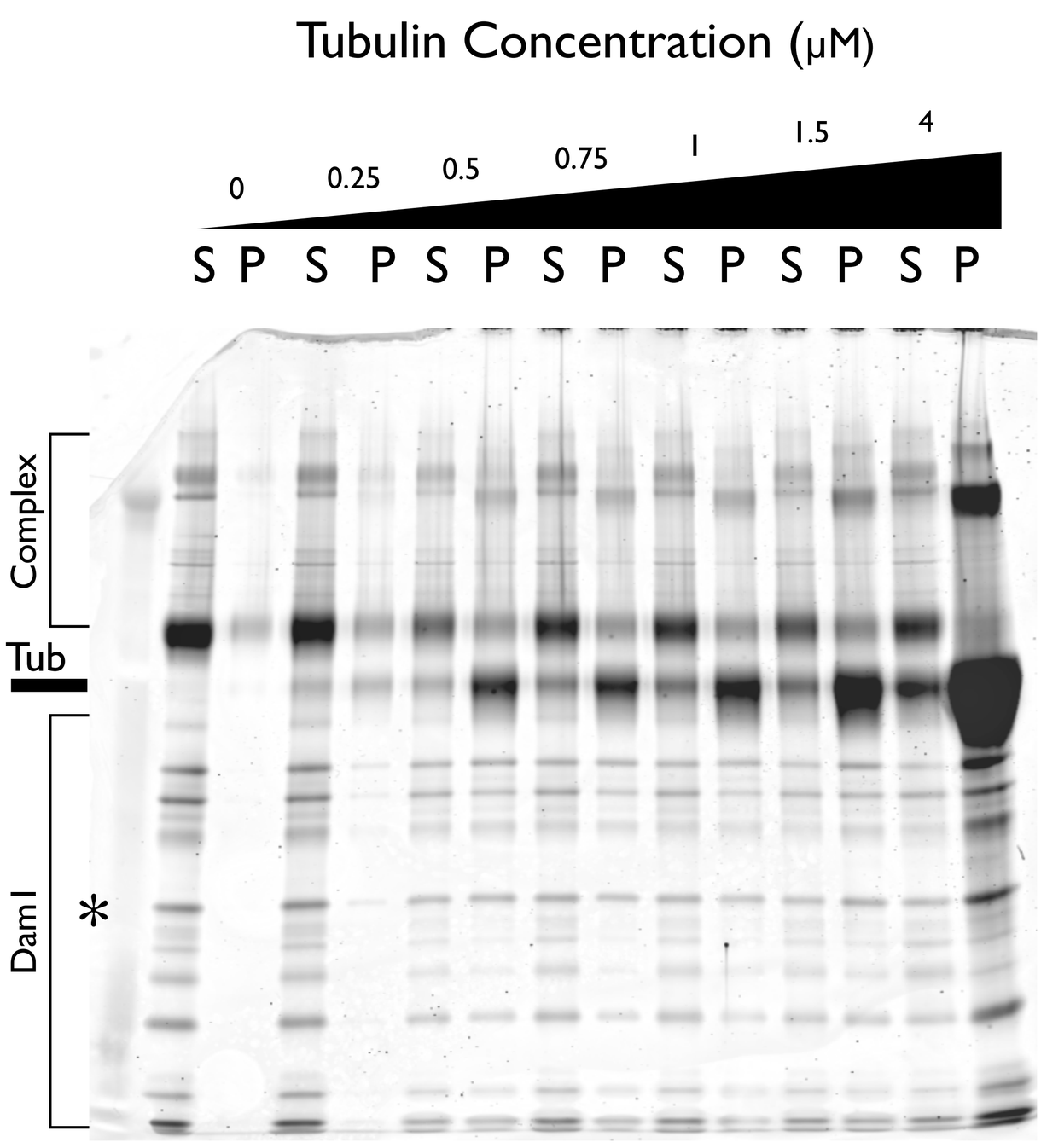




**Figure S4** – Representative gels used to calculate the binding curves shown in Figure 1d. The intensity of the tubulin and Dad2p bands (*) in each fraction were quantified to calculate the fraction of the Dam1 complex bound to either native or subtilisin-treated microtubules. Dad2p was chosen because its band sits far from other bands and therefore would give the most accurate intensity.

# WT Dam1 Ring FSC

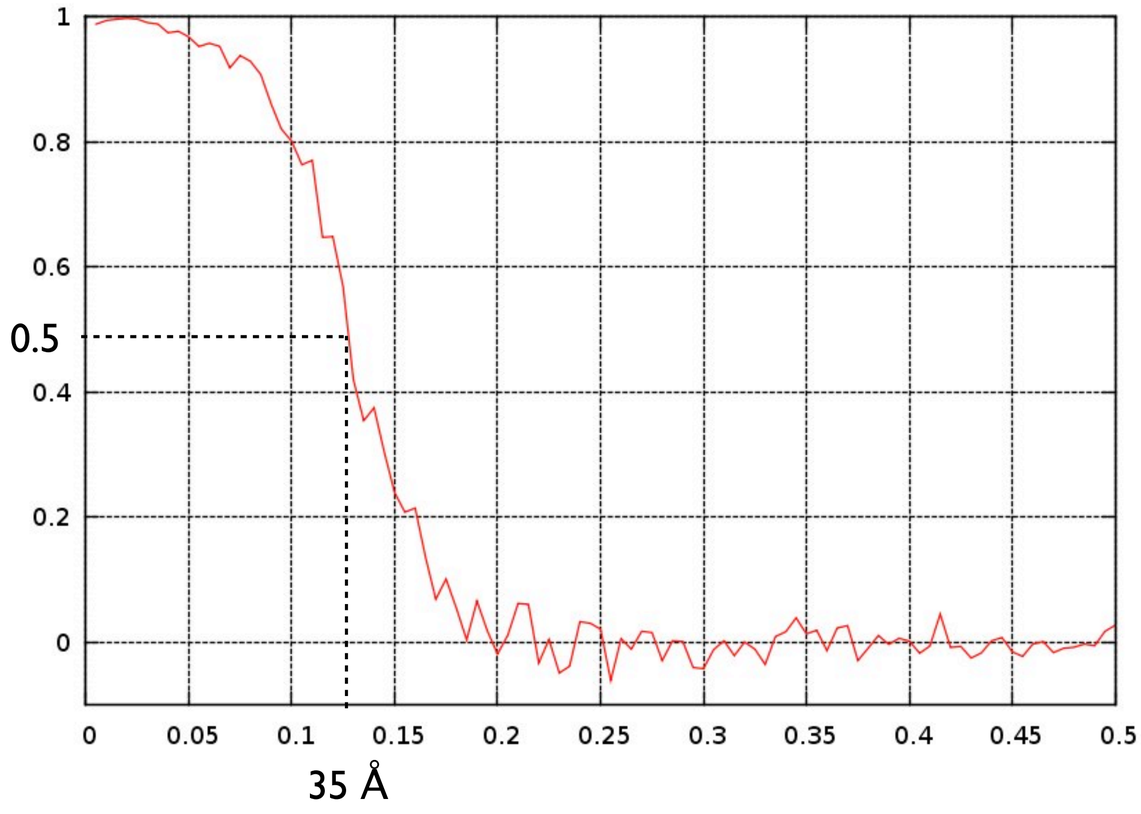



**Figure S5** – Fourier Shell Correlation (FSC) plots calculated for the two structures present in this study. From these graphs, and using the 0.5 FSC criteria, the resolution of the WT Dam1 ring obtained is 35 Å, and that for the ΔC Dam1 spiral is 38 Å.