**Figure S2:** A) The dependence of Mot1 ATPase activity on TBP concentration in reaction mixtures that contain 7 nM Mot1, 25 μM ATP and TBP whose concentration varied from zero to 250 nM. The $K_m$ for TBP of the observed saturation curve is ~76 nM. The $V_{max}$ for Mot1 ATPase activity is 35-fold over the basal rate. *Inset:* The dependence of Mot1 ATP hydrolysis rate on ATP concentration measured in reactions that contained 7 nM Mot1, 100 nM TBP and 1 to 200 μM ATP. The $K_m$ for ATP is 84 μM and $k_{cat} = 83$ moles ATP hydrolyzed per mol of Mot1. B) Reaction progress curves for Mot1-catalyzed ATP hydrolysis showing the percent ATP hydrolysis versus time. The reactions contained 25 μM ATP and 8 nM TBPC (diamonds), 7 nM Mot1 (circles) or 8 nM TBPC plus 7 nM Mot1 (squares). ATP concentration was determined spectrophotometrically using an extinction coefficient of 1.54 X 10$^4$ cm$^{-1}$ M$^{-1}$ at 260 nm. ATP hydrolysis by TBPC alone was not statistically significant above background. The rate of ATP hydrolysis by Mot1 alone was 0.13 s$^{-1}$ and in the presence of TBPC was 0.23 s$^{-1}$ (R = 0.99). Comparison of the rate of Mot1-catalyzed TBPC-DNA dissociation (Figure 4) with ATP hydrolysis measured under the same conditions yields a ratio of ~13 ATP molecules hydrolyzed for each TBPC-DNA complex dissociated. As Mot1 displays significant basal ATPase activity that was only weakly stimulated by TBPC under these conditions, ATP hydrolysis need not have been coupled to TBPC-DNA dissociation. Thus, this ratio represents an upper limit for the number of ATP molecules required per disruption event.
Figure S2