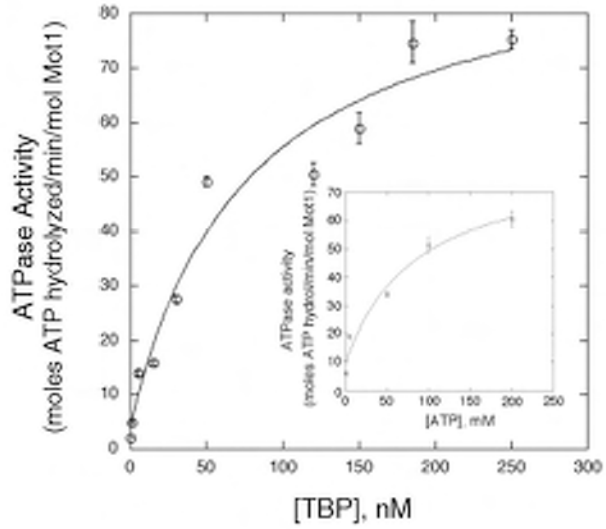
Figure S2: A) The dependence of Mot1 ATPase activity on TBP concentration in reaction mixtures that contain 7 nM Mot1, 25 μM ATP and TBP whose concentration varied from zero to 250 nM. The K_m for TBP of the observed saturation curve is ~76 nM. The V_{max} for Mot1 ATPase activity is 35-fold over the 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.

A


ATPase Activity
(moles ATP hydrolyzed/min/mol Mol1)
[TBP], nM
ATPase activity
[ATP], mM

B

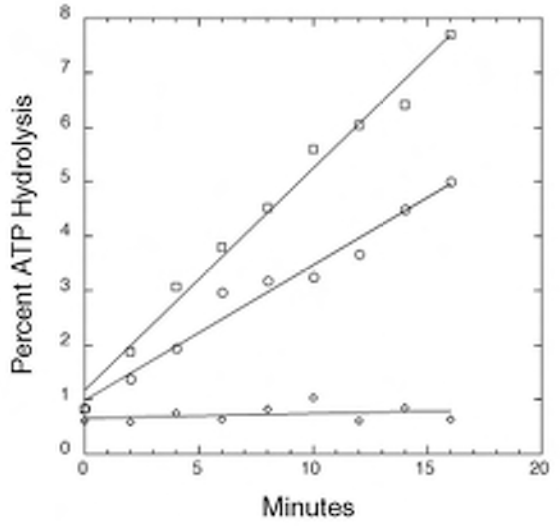

Percent ATP Hydrolysis
Minutes

Figure S2