



**Figure 1-figure supplement 1. GapR binding to supercoiled DNA in a magnetic tweezer experiment.**

- (A) No ligase control for GapR DNA topology assay. GapR was incubated with nicked (N) plasmid and treated identically as in Fig. 1A. Gel analysis of plasmid topology with supercoiled (S) and relaxed (R) standards. No digestion is observed at 2.5 - 5  $\mu\text{M}$  GapR due to GapR protection of DNA.
- (B) Analysis of ligation products from Fig. 1A with 2D-chloroquine electrophoresis. Migration of different plasmid forms are diagrammed (left): N, nicked; R, relaxed; L, linear; (-), negatively supercoiled; (+), positively supercoiled. Migration of the most abundant species indicated (asterisk).
- (C) Schematic of the microscope and flowcell setup in a magnetic tweezer (MT) experiment.
- (D) Behavior of naked DNA (left), DNA incubated with 1  $\mu\text{M}$  GapR (middle), and overlay (right) in a typical rotation-extension experiment. Data indicate mean  $\pm$  SEM, for 3 MT experiments.
- (E) Behavior of DNA incubated with 10 nM GapR in a typical rotation-extension experiment. Data indicate mean  $\pm$  SD,  $n = 200$  at each  $\sigma$ , in a single MT experiment (left) and mean  $\pm$  SEM compared to naked DNA (black), for 3 MT experiments (right).
- (F) Behavior of DNA incubated with 100 nM GapR in a typical rotation-extension experiment as in (E).
- (G) Rotation-extension curve (top left; data indicate mean  $\pm$  SD,  $n = 200$  at each  $\sigma$ ) and time course measurements for a single GapR binding experiment. Time courses indicate behavior of naked DNA (black) and DNA bound by 1  $\mu\text{M}$  GapR (orange) under positive supercoiling ( $\sigma = +0.03$ , top right), negative supercoiling ( $\sigma = -0.03$ , bottom left), and no supercoiling ( $\sigma = 0.0$ , bottom right).
- (H) Behavior of naked DNA (black), DNA incubated with 1  $\mu\text{M}$  GapR (orange) and after GapR washout (purple) in a typical rotation-extension experiment as in (E).
- (I) Time course measurements of naked DNA (black), DNA incubated with 1  $\mu\text{M}$  GapR (orange), and after GapR washout (purple) under negative supercoiling ( $\sigma = -0.03$ ).