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Quaternary Interactions And Supercoiling Modulate The Cooperative DNA Binding Of AGT Manana Melikishvili and Michael G. Fried

Supplemental Data.

Figure S1.



Figure S1. Determination of ensemble average binding affinities for 13nt, 13bp, 16nt and 16 bp DNAs. DNAs ($\sim 3 \times 10^{-9}$ M) were titrated with AGT protein ($0 \le [AGT] \le 5.2 \times 10^{-5}$ M) in buffer consisting of 10 mM Tris (pH 7.6), 1 mM DTT, 1mM EDTA, 100 mM NaCl. Free and bound DNA species were resolved by native electrophoresis (EMSA) as described for Fig. 2. Each data set is derived from 2 or more independent titrations. The smooth curves are fits of Eq. S1 to the data; the individual terms are defined in the main text. Results of these fits were within error the same as those for direct fits of [P] on v (Fig. 4); numerical values are summarized in Table 2.

$$\frac{v}{[P]} = K(1-sv) \left(\frac{(2\omega-1)(1-sv)+v-R}{2(\omega-1)(1-sv)} \right)^{s-1} \left(\frac{1-(s+1)v+R}{2(1-sv)} \right)^2 \left(\frac{N-s+1}{N} \right)$$

$$R = \left((1-(s+1)v)^2 + 4\omega v(1-sv) \right)^{1/2}$$
(S1)

Figure S2.



Figure S2. Scatchard plots for AGT binding to isolated pUC19 DNA topoisomers. Binding densities (v) were calculated from weight-average reduced molecular weights of AGT-DNA complexes, measured at sedimentation equilibrium, as described in Methods. Linking differences with respect to relaxed form DNA are indicated by the numbers near each curve. The smooth curves are fits of the long-chain version of the McGhee-von Hippel equation (Eq. S2) to the data. The terms in Eq. S2 are the same as those defined in the main text. Binding parameters (K, ω) returned by these analyses are given in Fig. 9.

$$\frac{v}{[P]} = K(1-sv) \left(\frac{(2\omega-1)(1-sv)+v-R}{2(\omega-1)(1-sv)} \right)^{s-1} \left(\frac{1-(s+1)v+R}{2(1-sv)} \right)^2$$
$$R = \left((1-(s+1)v)^2 + 4\omega v(1-sv) \right)^{1/2}$$
(S2)