Table 2: Equilibrium and Kinetic Constants for the Binding of the Egr-1 ZFD to Synthetic DNA Duplex Determined by Single-Molecule Counting[a]

Equilibrium Parameters	
assay	K_d (pM)
direct ligand[b]	13 (±3)
tracer ligand[c]	14 (±1)
competitive inhibitor[d]	10 (±3)

Kinetic Parameters	
assay	constant
association[e]	$k_{on} = 1.0\ (\pm 0.5) \times 10^9\ M^{-1}\ s^{-1}$
dissociation[f]	$k_{off} = 1.11\ (\pm 0.10) \times 10^{-3}\ s^{-1}$

[a] Average (±SD) of at least three independent determinations. Experimental conditions: 100 mM NaCl, 10 mM Tris at pH 8.0, 10 mM EDTA, 10 μM $ZnSO_4$, and 250 μg/mL BSA at room temperature. [b] Saturation titration of the protein with Cy5-EBS, as in Figure 7A. Data were fitted with eq 2. [c] Saturation titration of the protein with tracer Cy5-EBS/EBS (1:3), as in Figure 7C. Data fitted with a modified form of eq 2 yielded K_d^{app} equal to 3.5 (±0.2) pM. K_d was calculated from K_d^{app}/n, where n represents the fraction of DNA that is labeled with Cy5. [d] Saturation titration of the protein and 24 pM free Cy5-EBS with the competitor unlabeled EBS, as in Figure 7D. Data fitted with eq 3 yielded IC_{50} equal to 28 (±3) pM. K_d was calculated from IC_{50} using eq 4 and the 13 pM K_d value from the direct ligand titration assay. [e] Association time courses were initiated by the addition of Cy5-EBS to the protein. k_{on} was calculated using eq 6 from slopes of three independent determinations, as in Figure 8B. [f] The dissociation time course was initiated by the addition of a large molar excess of unlabeled EBS to the protein prebound to Cy5-EBS, as in Figure 9A. Data were fitted with monoexponential eq 1.