Supporting Information

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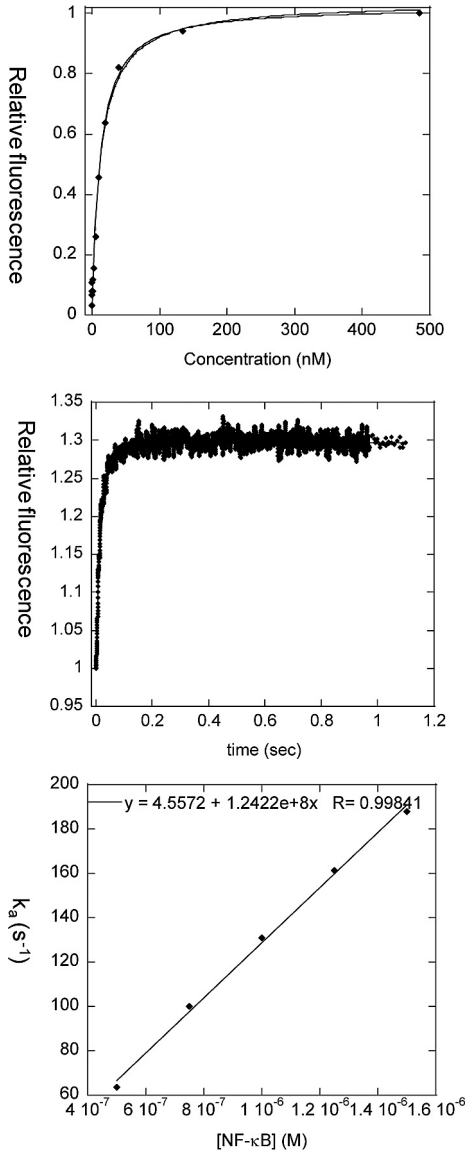

Fig. S1. (*A*) Equilibrium-binding measurements in which the concentration of pyrene DNA was held constant at 0.25 μM and increasing amounts of NF-κB(p50 $_{(19-363)}$/p65$_{(1-325)}$) protein were added. The concentration of the pyrene DNA was 5 nM, and the ratios of DNA:NF-κB used ranged from 100:1 to 1:100. The data were fit by 2 different equations: Michaelis-Menton m1*x/(m2+x), where m1 is the fluorescence amplitude and m2 is K_d, and the quadratic equation m1*((m2+m3+x)−((m2+m3+x)*(m2+m3+x)−4*m3*x)^(1/2))/(2*m3), where m1 is the maximum fluorescence; m2 is K_d, and m3 is the NF-κB concentration. As expected, the fits gave identical results because the experiment was performed at low concentrations near the K_d. (*B*) Stopped-flow kinetic measurements of the binding of pyrene DNA to NF-κB(p50$_{(19-363)}$/p65$_{(1-325)}$) were performed in which the pyrene DNA concentration was fixed at 0.25 μM and different concentrations of NF-κB were added. The equation used to extrapolate the on-rate was Y = m1+m2*exp(−m3*x), where m1 is maximum florescence value, m2 is the amplitude of fluorescence, and m3 corresponds to the K_D. The figure shows a representative trace in which the concentration of NF-κB was 0.5 μM. In total, 9 concentrations were analyzed, and each concentration was analyzed 6 separate times. (*C*) The observed association rates from the experiments in (*B*) are plotted vs. NF-κB, and the resulting line is fit to a linear equation where the intercept is k_{off} and the slope is k_{on}, yielding values of k_{on} of 1.2 $\pm$ 0.070 $\times$ 10^8 $M^{-1}s^{-1}$ and k_{off} of 4.56 $\pm$ 0.94 s^{-1}. The ratio k_{off}/k_{on} provided K_d of 37 $\pm$ 3.9 nM, which, as expected, was not consistent with the value obtained with the equilibrium-binding measurements because the concentration of the pyrene-DNA is above the K_d.

Table S1. Binding of NF-κB homo- and heterodimers to various κB DNA sites

NF-κB	DNA	k_a ($\times 10^6$ $M^{-1}s^{-1}$)	k_d ($\times 10^{-3}s^{-1}$)	$K_{D,BIA}$ ($\times 10^{-9}M^{-1}$)
Heterodimer				
$p50_{(39–376)}/p65_{(1–325)}$	IgκB	1.2 ± 0.2	7.3 ± 0.7	6.3 ± 0.7 (25°C)
$p50_{(39–376)}/p65_{(1–325)}$	IgκB	1.2 ± 0.3	17 ± 0.5	14.7 ± 0.6 (37°C)
$p50_{(39–376)}/p65_{(1–325)}$	MIP2	0.52	5.1	9.9
$p50_{(39–376)}/p65_{(1–325)}$	RANTES	2.0	7.4	3.7
$p50_{(39–376)}/p65_{(1–325)}$	UK	1.1	33.7	31.9
$p50_{(39–376)}/p65_{(1–325)}$	IFN	2.1	13.7	6.7
Homodimer				
$p65_{(19–325)}/p65_{(1–325)}$	IgκB	0.015	104	7370
$p65_{(19–325)}/p65_{(1–325)}$	MIP2	0.59	13.2	22.3
$p65_{(19–325)}/p65_{(1–325)}$	RANTES	0.63	25.9	41.2
$p65_{(19–325)}/p65_{(1–325)}$	UK	0.66	80.0	124
$p65_{(19–325)}/p65_{(1–325)}$	IFN	1.08	22.9	21.2

Binding was done in 20 mM Tris (pH 7.5), 150 mM NaCl, 10% glycerol.

Table S2. Binding of NF-κB($p50_{(19–363)}$/$p65_{(1–325)}$) to IκBα and rates of dissociation

A. Binding of NF-κB($p50_{(19–363)}$/$p65_{(1–325)}$) at 37 °C to truncated IκBαs and rates of dissociation

DNA	IκB	k_a ($\times 10^6$ $M^{-1}s^{-1}$)	k_d ($\times 10^{-3}s^{-1}$)	K_D (nM)	Active dissociation rate ($\times 10^5$ $M^{-1}s^{-1}$)
IgκB	IκBα (67–317)	3.3 ± 0.6	0.14 ± 0.055	0.045 ± 0.01	9.3
IgκB	IκBα (67–287)	3.7 ± 0.1	0.15 ± 0.014	0.039 ± 0.003	9.4 ± 1.4
IgκB	IκBα (67–281)	0.9 ± 0.3	2.2 ± 0.075	2.47 ± 0.16	5.9
IgκB	IκBα (67–275)	1.9 ± 0.1	39.8 ± 2.2	20.7 ± 0.1	1.4

B. Binding of NF-κB($p50_{(19–363)}$/$p65_{(1–325)}$) at 37 °C to mutants of IκBα and rates of dissociation

DNA	IκBα (67–287)	k_a ($\times 10^6$ $M^{-1}s^{-1}$)	k_d ($\times 10^{-3}s^{-1}$)	K_D (nM)	Rel. K_D	Active dissociation rate ($\times 10^5$ $M^{-1}s^{-1}$)
IgκB	C186P,A220P	3.3 ± 0.2	0.16 ± 0.02	0.048 ± 0.008	1	7.3 ± 0.4
IgκB	Q111G,C186P,A220P	0.98 ± 0.09	0.036 ± 0.02	0.035 ± 0.019	0.9	6.4 ± 1.8
IgκB	Y254L/Q255H	0.67 ± 0.12	2.55 ± 0.11	3.9 ± 0.15	100	6.0
IgκB	Q111G				2[a]	7.4 ± 0.6
IgκB	Y254L,T257A				30[a]	2.8

[a]Relative K_D determined from binding to NF-κB($p50_{248–350}$/$p65_{190–321}$)