The kinetics of CR complex formation between $E\sigma^{70}$ RNAP and λP_R promoter DNA were studied as a function of urea (0, 0.21, 0.42, 0.63 m) at 17.1 °C in BB^{dissoc}_{urea}, the same conditions used for the dissociation reactions in the main text. Reactions were followed by nitrocellulose filter binding following manual mixing of RNAP and promoter DNA, as described (*1*). Most of these reactions (except for those at high [RNAP] in the absence of urea) are reversible, reaching an equilibrium distribution of free RNAP and DNA, I_1, and CR complexes, instead of reacting to completion. These reactions are plotted as θ_t^{CR} versus time (as represented in Figures S1A and B for 0 and 0.42 m urea) and analyzed as decays to equilibrium where β_{CR} is a single-exponential relaxation rate constant which must be corrected for the contribution of the dissociation reaction to the relaxation kinetics to obtain the composite forward rate constant, α_{CR} (*1*):

$$\theta_t^{CR} = \theta_{eq}^{CR} (1 - e^{-\beta_{CR} t}) \quad \text{(S1)}$$

$$\alpha_{CR} = \beta_{CR} - k_d = \beta_{CR}(\theta_{eq}^{CR}) \quad \text{(S2)}$$

where θ_{eq}^{CR} is the value of θ_t^{CR} at equilibrium (as $t \rightarrow \infty$) and k_d is for the same reaction conditions as β_{CR}. For irreversible reactions (those at the highest [RNAP] at 0 m urea in BB^{dissoc}_{urea}, as well as those in the main text), θ_{eq}^{CR} is unity (at equilibrium, all promoter DNA is in the form of CR complexes, I_2 and RP_o) and $\beta_{CR} = \alpha_{CR}$.

For the series of experiments at the same [RNAP], the equilibrium fraction of promoter DNA present as CR complexes (the plateau level) decreases with increasing [urea]: at 5 nM RNAP, θ_{eq}^{CR} decreases from ~0.8 at 0 m urea to ~0.6 at 0.42 m urea (Figures S1A and B). Thus, as [urea] increases, conversion to CR complexes is disfavored and the amount of free DNA and RNAP and I_1 at equilibrium increases. For

the conditions of these experiments, k_d ranges from ~20% of β_{CR} at 6.7 nM RNAP and 0.63 m urea to <1% of β_{CR} at 70 nM RNAP and 0 m urea.

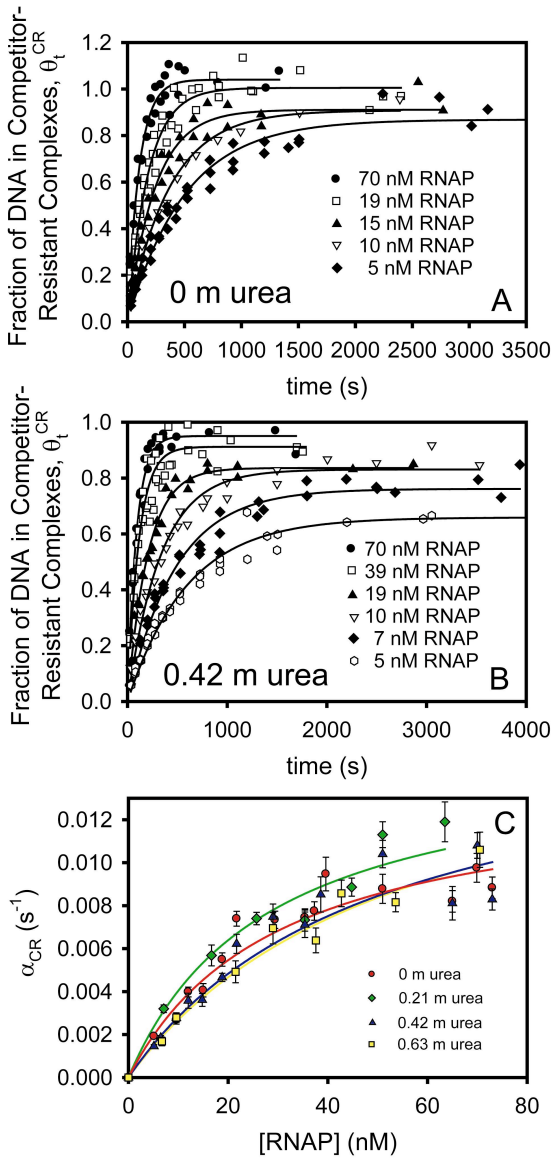
Figure S1C plots $\alpha_{CR} = \beta_{CR} - k_d$ versus [RNAP] for all experiments as a function of urea concentration. At each urea concentration, α_{CR} increases hyperbolically with increasing [RNAP], as expected from eq 5 in the main text. Even though K_1 for R + P ⇌ I_1 is a maximum at ~17 ºC (*1*), K_1 turns out to be is insufficiently large at the salt concentration of these experiments to obtain full occupancy of promoter DNA even in the absence of urea at low [RNAP]. Consequently, an additional level of uncertainty is introduced into the determination of K_1 and k_2 from the hyperbolic fits to α_{CR} versus [RNAP] at each ureaconcentration (see Table S1). Under these conditions (17.1 ºC in BB^{dissoc}_{urea}), the equilibrium constant K_1 appears to decrease non-monotonically ($\partial \ln K_1/\partial m_{urea} = -0.7 \pm 2\ m^{-1}$) and k_2 may increase slightly with increasing urea concentration, although the large uncertainty makes this apparent effect insignificant ($\partial \ln k_2/\partial m_{urea} = 0.3 \pm 0.3\ m^{-1}$).

Table S1. Values of K_1 and k_2 at 0, 0.21, 0.42, and 0.63 m urea at 17.1 °C in BB_{urea}^{dissoc}.

	K_1 (M^{-1})	k_2 (s^{-1})
0 m urea	$(3.3 \pm 0.6) \times 10^7$	$(1.4 \pm 0.2) \times 10^{-2}$
0.21 m urea	$(3.6 \pm 0.9) \times 10^7$	$(1.5 \pm 0.2) \times 10^{-2}$
0.42 m urea	$(2.0 \pm 0.4) \times 10^7$	$(1.7 \pm 0.2) \times 10^{-2}$
0.63 m urea	$(1.8 \pm 0.4) \times 10^7$	$(1.8 \pm 0.3) \times 10^{-2}$
$\left(\frac{\partial \ln(K \text{ or } k)}{\partial m_{urea}}\right)_{m_4,m_5}$	-0.7 ± 2 m^{-1}	0.3 ± 0.3 m^{-1}

Figure S1: Effects of urea on the reversible kinetics of formation of CR complexes between RNAP holoenzyme and λP_R promoter DNA. Nitrocellulose filter binding data for the fraction of DNA in the form of CR complexes (θ_t^{CR}) are shown as a function of time after manually mixing with RNAP in BB_{urea}^{dissoc} at 17.1 °C. Solid lines are fits of the data to eq S1 to determine the relaxation rate constant β_{CR}. (A) 0 m urea. (B) 0.42 m urea. (C) Dependence of the forward rate constant α_{CR} on [RNAP] at 0, 0.21, 0.42, and 0.63 m urea. α_{CR} (obtained from β_{CR} and k_d; eq S2) is plotted versus [RNAP] for the data of (A) and (B) and corresponding experiments at additional urea concentrations. Solid lines are fits of the data to eq 5 in the main text to determine K_1 and k_2 at each urea concentration.

Figure S1



(1) Saecker, R. M., Tsodikov, O. V., McQuade, K. L., Schlax, P. E., Jr., Capp, M. W., and Record, M. T., Jr. (2002) Kinetic studies and structural models of the association of E. coli sigma(70) RNA polymerase with the lambdaP(R) promoter: large scale conformational changes in forming the kinetically significant intermediates. *J Mol Biol 319*, 649-71.