Supplemental Information

Cooperative stabilization of *Mycobacterium tuberculosis rrnA*P3 promoter open complexes by RbpA and CarD.

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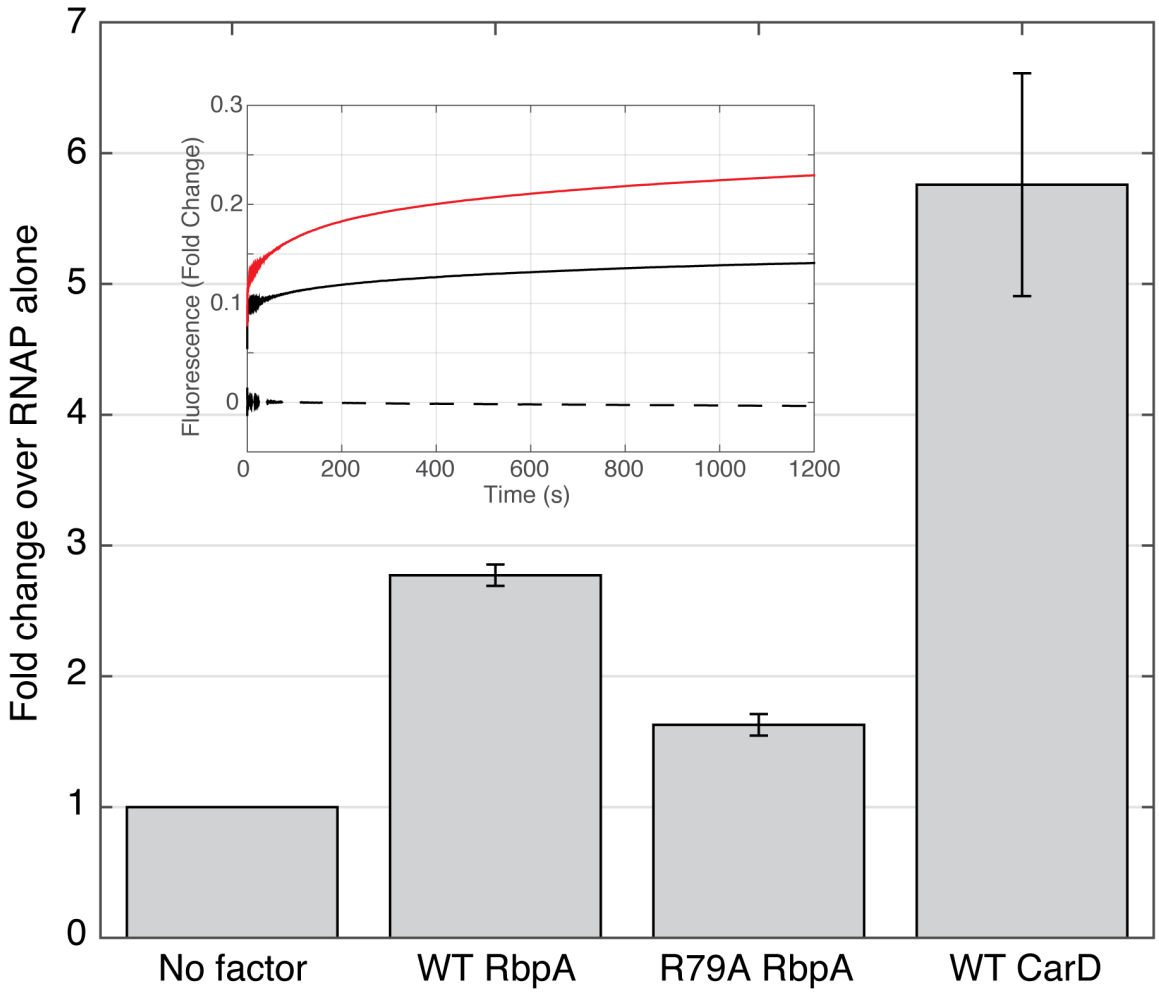
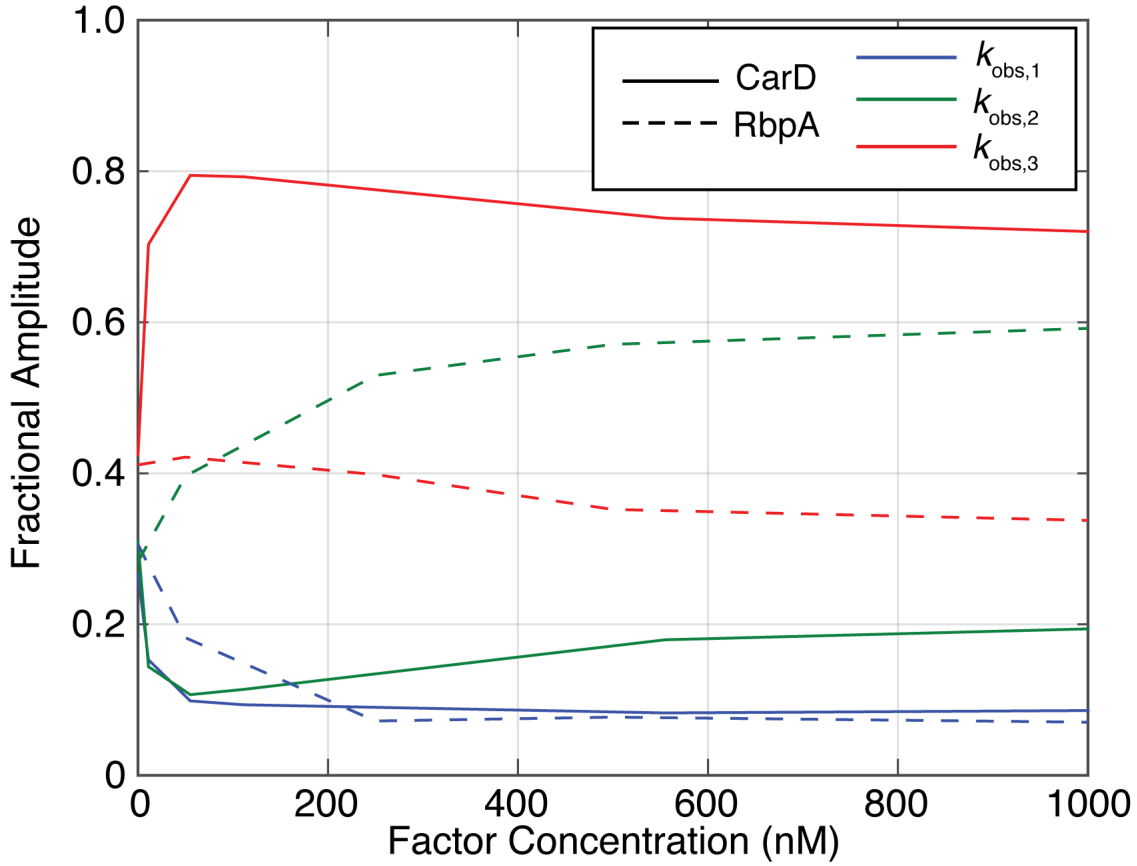
Supplemental Sequence

Non-template strand of 150 bp *Mtb rrnA*P3 promoter fragment used in this study (genome coordinates 1471577 – 1471727). The -10 and -35 boxes (blue), +1 start site (red), and +2 T modified with a C6-amine and labeled with Cy3-NHS (green) are indicated {GonzalezyMerchand:1996-va}.

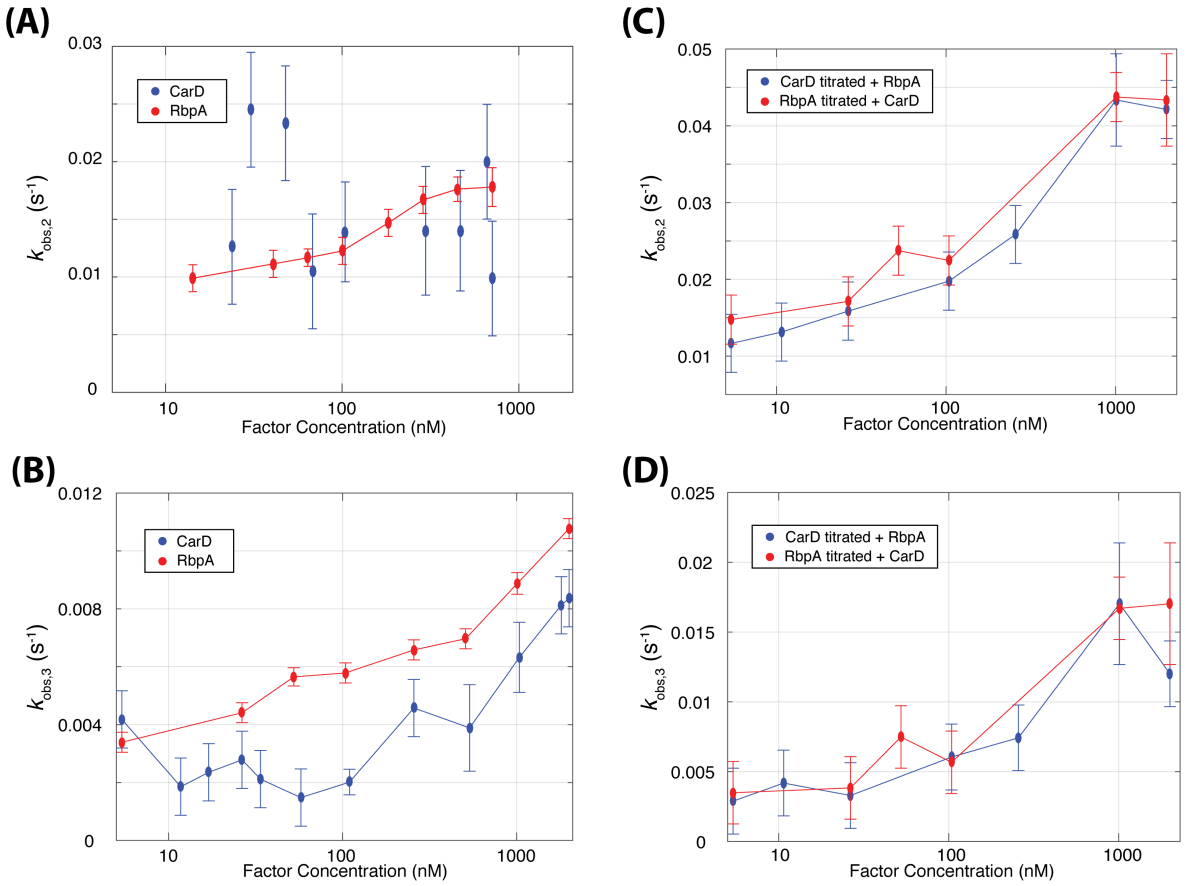
GGCGACGGTCACCTATGGATATCTATGGATGACCGAACCTGGTCTTGACTCCATTGCCG-
GATTTGTATTAGACTGGCAGGGTTGCCCCGAAGCGGGCGGAAACAAGCAAGCGTGTTGTTTGA-
GAACTCAATAGTGTGTTTGGTGGTTTCA



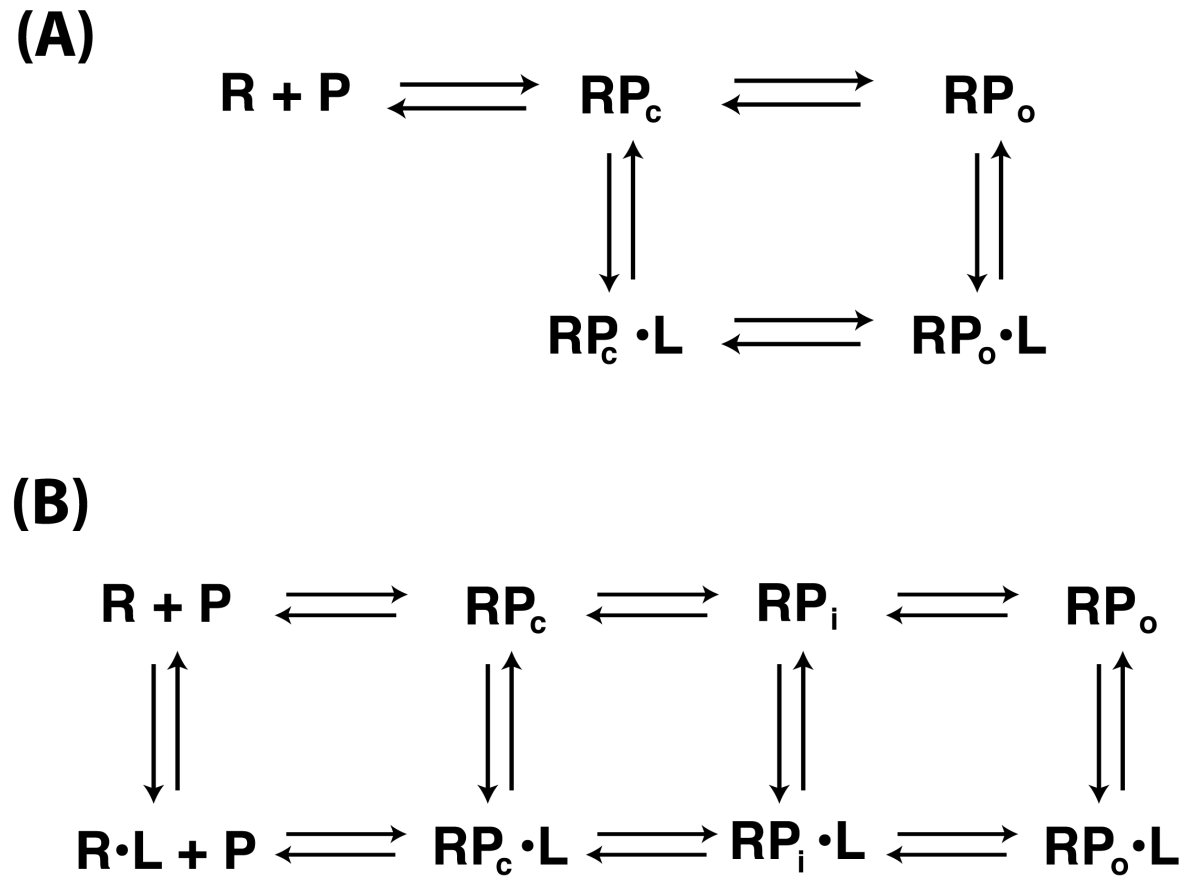
Supplemental Figure 1: RbpA R79A mutant is defective in open complex stabilization. The fluorescence fold change over RNAP alone is shown for 2 μM WT RbpA, 2 μM R79A RbpA, and 1 μM WT CarD. Error bars represent standard error of the mean. The inset shows the raw time traces for 10 nM DNA alone (black dashed), 225 nM RNAP and 10 nM DNA (black), and 225 nM RNAP, 10 nM DNA, and 2 μM R79A RbpA (red).



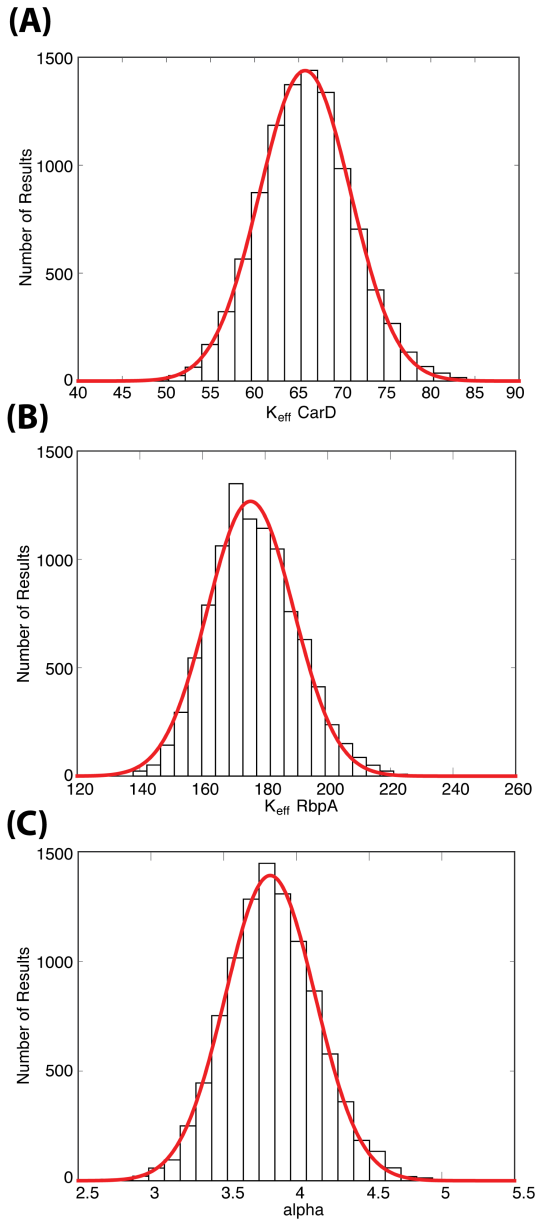
Supplemental Figure 2: Fractional amplitude dependence on factor concentration for each factor individually. Triple exponential fits to RbpA and CarD time courses exhibit different fractional amplitudes. For both RbpA (dashed lines) and CarD (solid lines), the amplitude of the fastest phase ($k_{obs,1}$, blue) drops below 10% as the concentration of each factor approaches 1 μM. Unlike CarD, the fractional amplitudes of RbpA fits are not dominated by the slowest phase ($k_{obs,3}$, red); instead, the intermediate phase ($k_{obs,2}$, green) exhibits a more significant contribution.



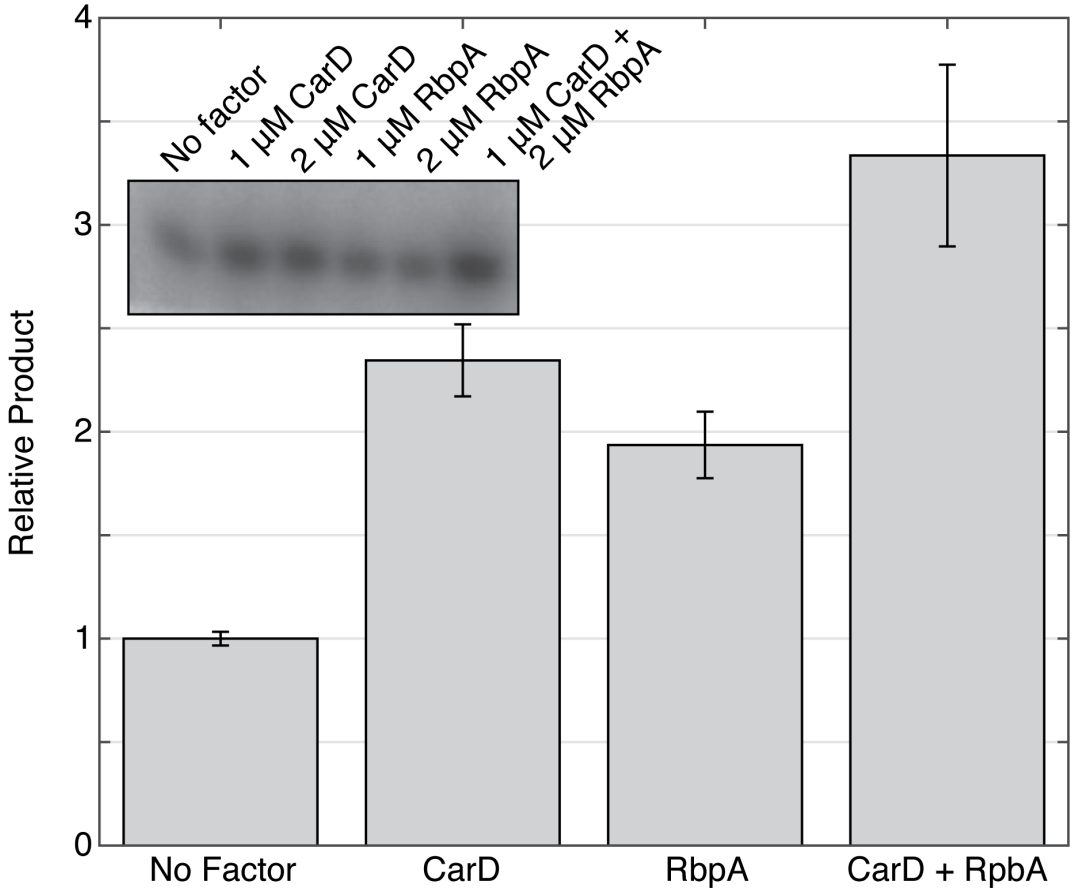
Supplemental Figure 3: Concentration dependence of the observed rates with factor concentration plotted in log scale. (A) The second observed rate ($k_{obs,2}$) as a function of the concentration of CarD (blue) and RbpA (red). **(B)** The third observed rate ($k_{obs,3}$) as a function of the concentration of CarD (blue) and RbpA (red). **(C)** The second observed rate ($k_{obs,2}$) as a function of the concentration of CarD in the presence of 2 μM RbpA (blue) and of the concentration of RbpA in the presence of 1 μM CarD (red). **(D)** The third observed rate ($k_{obs,3}$) as a function of the concentration of CarD in the presence of 2 μM RbpA (blue) and of the concentration of RbpA in the presence of 1 μM CarD (red).



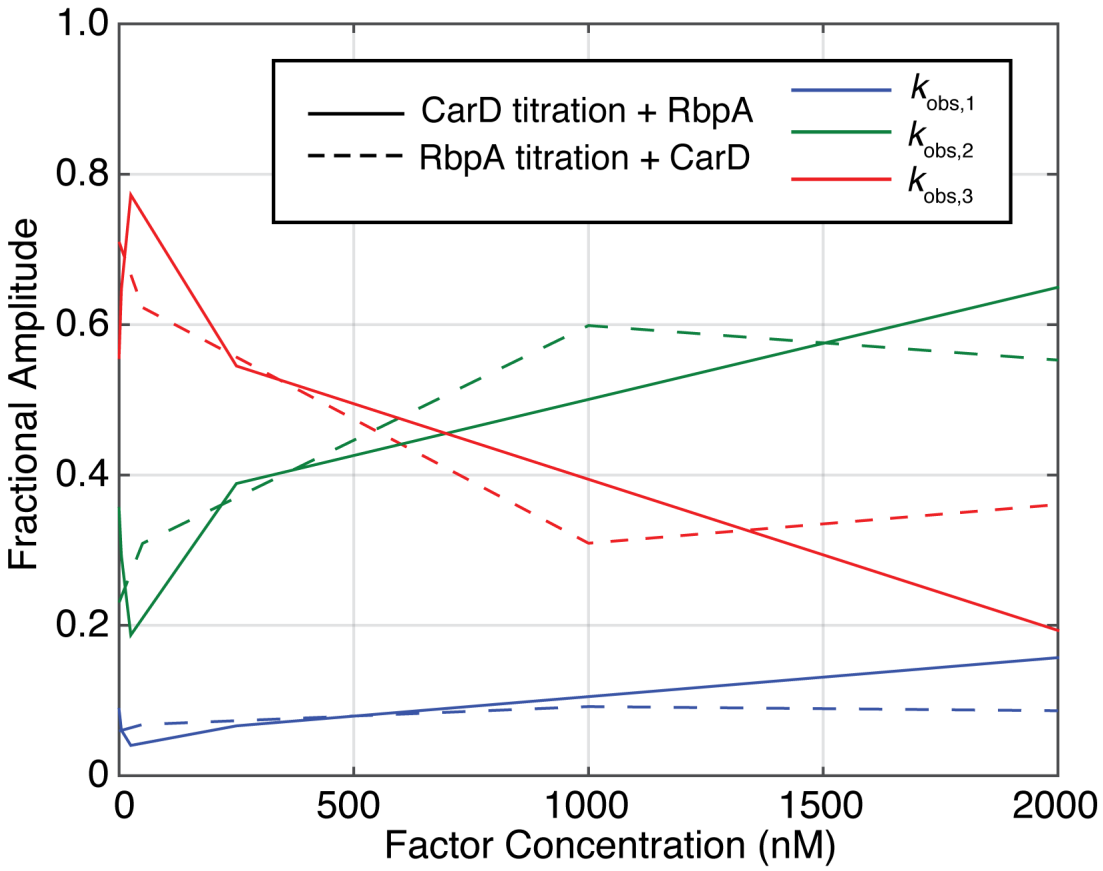
Supplemental Figure 4: Models for factor mechanisms. (A) The binding of RNAP polymerase (R) to the promoter (P) creates the closed complex (RP_c) which can isomerize to open complex (RP_o). A ligand (L) that may represent CarD or RbpA or any other factor, may bind to either RP_c or RP_o. Isomerizations between RP_c and RP_o may also occur in their ligand-bound states ($RP_c{\cdot}L$ and $RP_o{\cdot}L$). **(B)** As described in the text, the observed kinetics dictate that a more complicated kinetic scheme be considered to account for the data. Here, we illustrate two possible additions to the scheme to consider. The first is the addition of the state (R·L + P) which represents the association of either CarD or RbpA to free, promoter-unbound holoenzyme subunits. The R here can be further expanded to specifically include contributions from core-, sigma-, and holoenzyme-bound complexes. The second is the explicit representation of intermediates (RP_i) between RP_c and RP_o as well as the possible complexes between these intermediates and CarD or RbpA ($RP_i{\cdot}L$).



Supplemental Figure 5: Distributions of global fit parameters for cooperative binding model. Data points were randomly selected with replacement and then fit with a cooperative binding model with 3 parameters. The distributions of the affinity (K_{eff}) of **(A)** CarD and **(B)** RbpA alone to polymerase bound promoter complexes are shown along with **(C)** the cooperativity factor alpha. These distributions were fit with a gaussian to determine the both the mean and uncertainty for each parameter based on the data.



Supplemental Figure 6: Three nucleotide aborted transcription. The relative amount of 3-nt product compared to that generated by MboRNAP holoenzyme alone is shown as a function of the addition of CarD (2 μM), RbpA (2 μM), or both factors. Error bars represent standard errors of the mean from 8 replicates. The inset shows a gel from one of the replicates and includes lanes for each factor at both 1 μM and 2 μM as well as the combination of both factors at saturating concentrations.



Supplemental Figure 7: Fractional amplitude dependence on factor concentration in the presence of both factors. The titration of RbpA in the presence of 1 μM CarD (dashed lines) and the titration of CarD in the presence of 2 μM RbpA (solid lines) reveal similar trends in the fractional amplitudes of the fastest ($k_{obs,1}$, blue), intermediate ($k_{obs,2}$, green), and slowest ($k_{obs,3}$, red) phases. Interestingly, CarD titrations performed in the presence of RbpA no longer show a dominance of the slowest phase ($k_{obs,3}$).