Supplemental Information

CarD stabilizes mycobacterial open complexes via a two-tiered mechanism.

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SUPPLEMENTAL METHODS

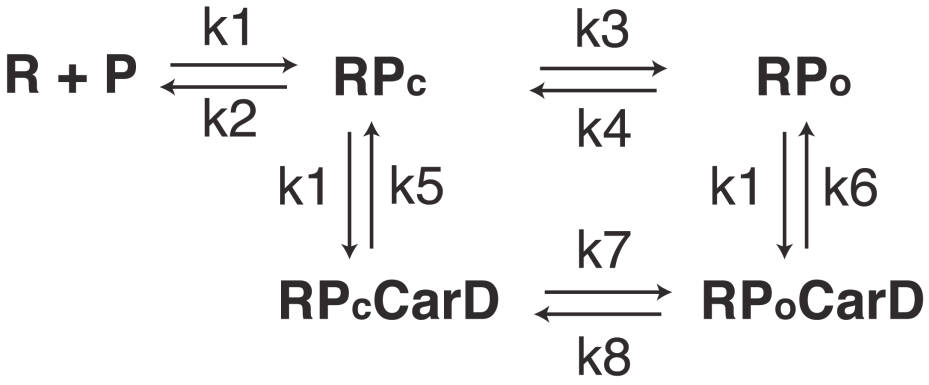
Template sequence

The full non-template strand sequence of the 150 bp *rrnA*P3 DNA template used in the fluorescent assay. The italics/underline indicate genomic sequence and the green, blue, red, and orange type indicate the -35 box, -10 box, start-site, and Cy3 label position respectively.

AAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTC*GTCTTGACTCCATTGCCG GATTTGTATTAGACTGGCAGGGTTG*AAGCTTATGTATCACCTGACGTCATGATGAACTC AGAAGTGAAACTACGTATATCCGATGGTAGTG

Kinetic simulations

The model as presented in the paper was constructed with five states and seven independent rates. The three effective bimolecular on rates were linearly dependent on the concentrations of the binding partners and were all set to be equal. Furthermore, the dissociation rate of CarD from open complex was calculated to satisfy detailed balance. Specifically, k6 = (k3*k8*k5)/(k4*k7).



The values of the rates used to generate the plots in the main text are as follows: k1 = 0.3 $s^{-1}nM^{-1}$, k2 = 75 s^{-1}, k3 = 0.0005 s^{-1}, k4 = 0.004 s^{-1}, k5 = 400 s^{-1}, k6 = 2.0 s^{-1}, k7 = 0.0125 s^{-1}, k8 = 0.0005 s^{-1}.

Measurements of protein concentration *in vivo*

Five mL of 4 replicate cultures of *Mycobacterium smegmatis* Mc^2155 in exponential growth phase were collected and the cells were pelleted and lysed in 500 µl of NP-40 (10 mM sodium phosphate, pH 8.0, 150 mM NaCl, and 1% Nonidet® P-40) by bead beating (FastPrep; MP Bio). Before lysing, the OD(λ_{600}) was measured for each culture and the conversion of OD (λ_{600}) of 1 corresponds to 5 x 10^8 cells/ml was used to determine the number of cells in each sample. The total protein concentrations of the lysates were also measured by BCA assay (Pierce). Undiluted, 1:5 dilution, and 1:10 dilution of cell lysates were run on a denaturing polyacrylamide gel adjacent to lanes containing 0.05, 0.1, 0.5, or 1.0 pmol each of *M. smegmatis* CarD, *M. bovis* RNAP core, and *M. bovis* RNAP σ^A purified protein. Following SDS PAGE, western blot analyses were performed and CarD, RNAP σ, and RNAP β were detected using mouse monoclonal antibodies specific for CarD (clone 10F05; Memorial Sloan-Kettering Cancer Center Monoclonal Antibody Core Facility), RNAP σ (clone 2G10, Neoclone, Madison, WI), and RNAP β (clone 8RB13; Neoclone, Madison, WI), respectively. Western blots were imaged and quantified using an Odyssey CLX imaging system (LI-COR). Standard curves for CarD, RNAP σ^A, and RNAP β were generated and used to determine the pmol/lane for each protein in the lysates. The molecules/cell calculation assumes 100% cell lysis and a cell count given by OD. The conversion of molecules/cell to protein concentration assumes a cell volume of 4 μM^3.

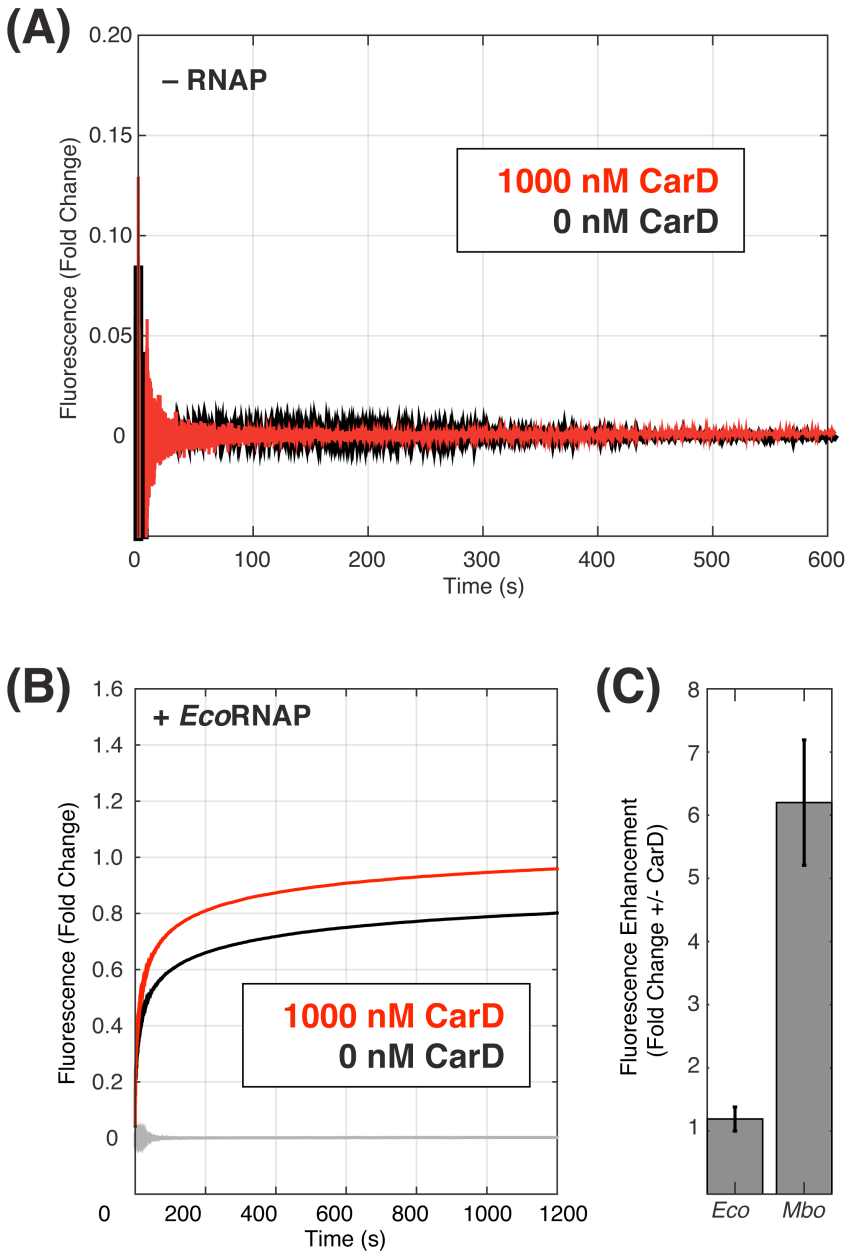


Figure S1: CarD-dependent fluorescent enhancement depends on *Mbo*RNAP. (A) The trace of 1 μM CarD in the absence of RNAP shows no fluorescent enhancement. **(B)** In the presence of 21 nM *Eco*RNAP, the addition of 1 μM CarD shows only a slight fold change relative to *Eco*RNAP alone. **(C)** The magnitude of the fluorescence fold change when CarD is added to concentrations of *Eco*RNAP and *Mbo*RNAP in the middle of their respective concentration dependences. Specifically, 21 nM *Eco*RNAP is compared to 225 nM *Mbo*RNAP. This comparison shows the effect of CarD on the respectively polymerases under conditions where they alone generate about half open complex. This demonstrates that CarD has a much greater effect on *Mbo*RNAP.

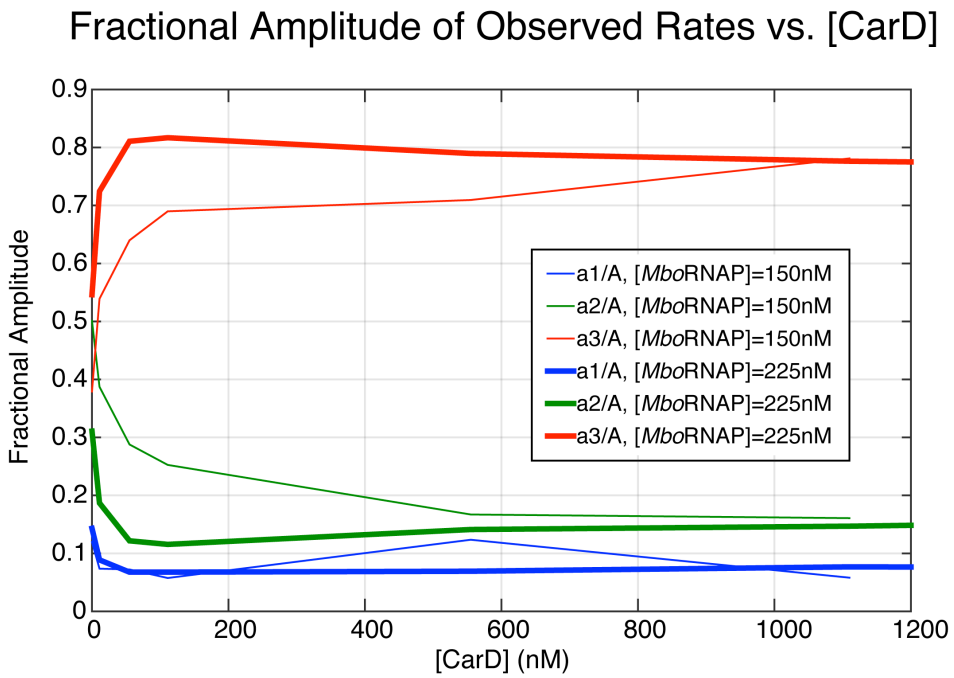


Figure S2: Fractional amplitudes of the three observed rates. Each observed rate (k^1_{obs}, k^2_{obs}, k^3_{obs}) obtained from the triple exponential fit carries an amplitude (a1, a2, a3) which describes that phase's contribution to the total fluorescent enhancement signal. The majority of the increase of the overall opening signal (fluorescent enhancement) as a function of CarD concentration is mostly due to increases in the amplitude of slowest observed rate (a3). This is shown by plotting the fractional amplitude of the rates (i.e. a1/A where A is the total amplitude of the trace) as a function of CarD concentration. The fraction of the total signal accounted for by the amplitude of the slow phase (red) increases while it decreases for the faster phases (blue and green). This is true for both concentrations of *Mbo*RNAP tested (150 nM and 225 nM).

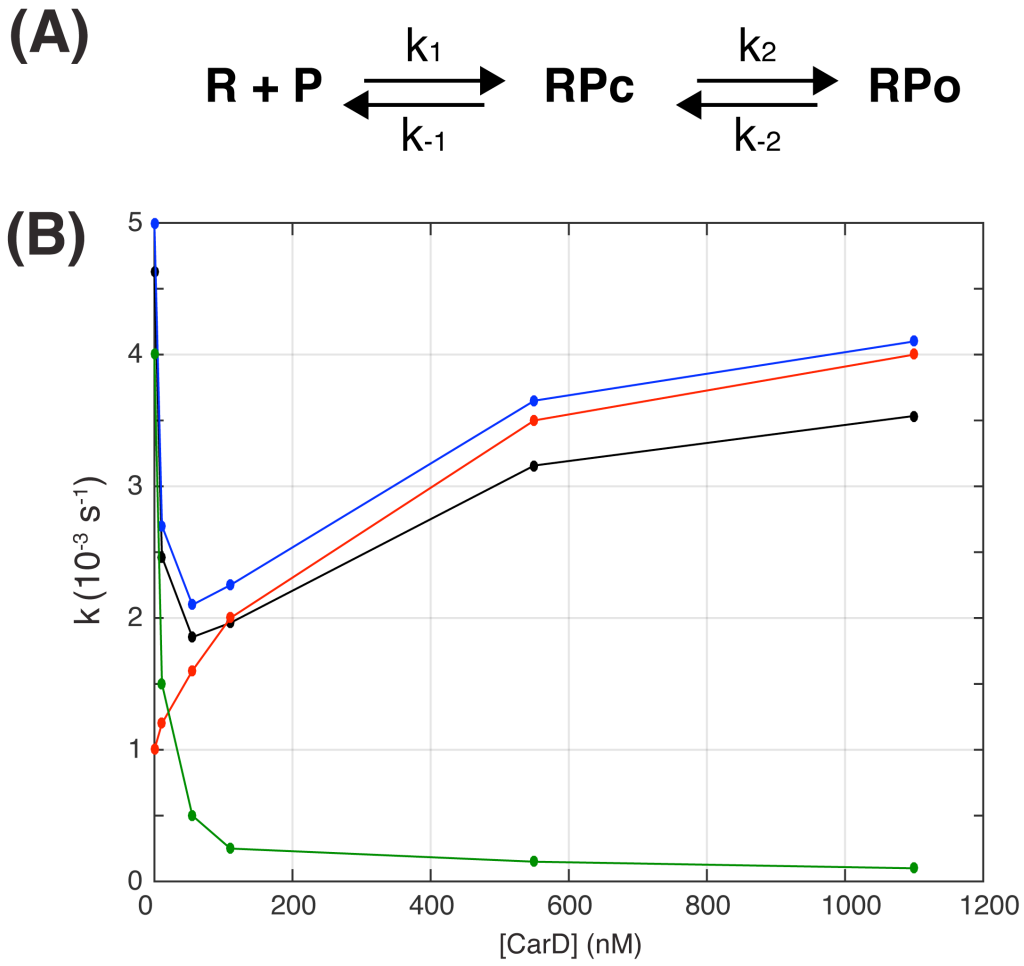


Figure S3: Observed rates in a three state example. (A) A simpler model is used to demonstrate the effect of microscopic rate constants on observed rate. In this model k_1 and k_{-1} describe the association and dissociation of RNAP with DNA respectively. Similarly, k_2 and k_{-2} describe the opening and closing rates respectively. **(B)** The CarD effect is captured simply by running the simulation with different combinations of k_2 and k_{-2} to represent different concentrations of CarD. The dependence on CarD concentration for k_2 (red) and k_{-2} (green) show the values input into the simulation used to generate time-dependent curves of the equilibration of open complex concentration (not shown). These curves are fit by an exponential to extract a measure of the observed rate (black). The concentration dependence of the measured observed rate (black) closely follows the trend of the sum of the two rates controlling the equilibrium between open and closed complex, $k_2 + k_{-2}$ (blue). This example demonstrates how combinations of microscopic rate constants lead to observed rates and how trends in observed rates can be analyzed to extract mechanistic information.

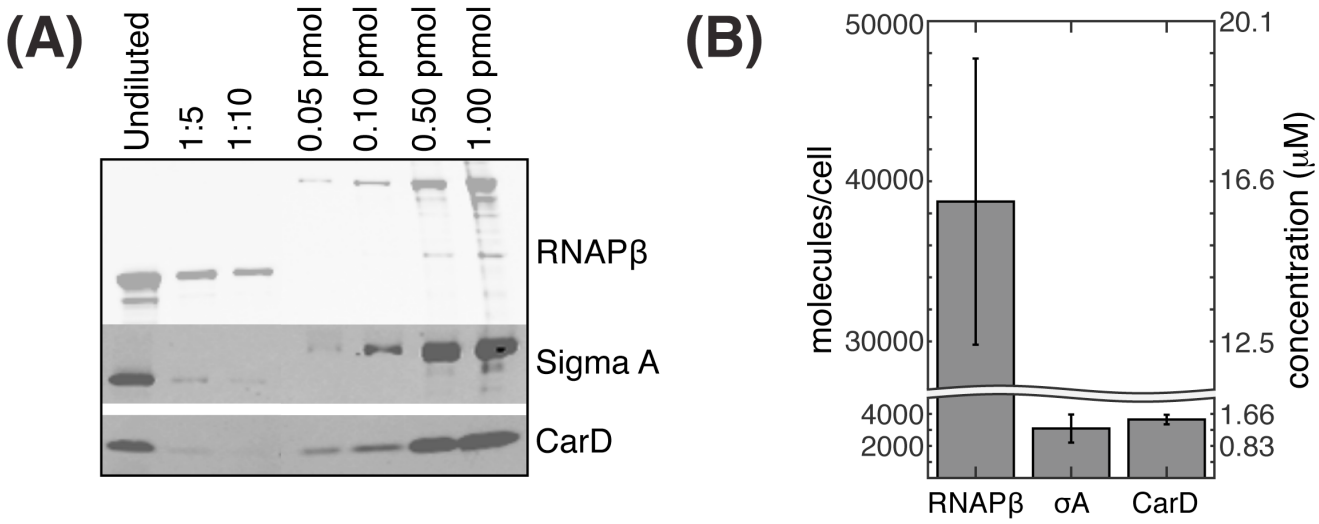


Figure S4: Concentrations of RNAP and CarD *in vivo*. (A) Western blot used for quantitation of protein levels found in lysates of *M. smegmatis* during logarithmic growth. Dilutions of lysate are shown next to lanes loaded with known amounts of each protein. **(B)** Bar graph shows the mean ± SEM of for each protein in the 4 replicates. The number of molecules/cell and the equivalent concentration are shown on the 2 y-axes. The conversion of the raw data to molecules/cell assumes 100% lysis of the collected cells and the conversion to concentration assumes a cell volume of 4 μm^3.

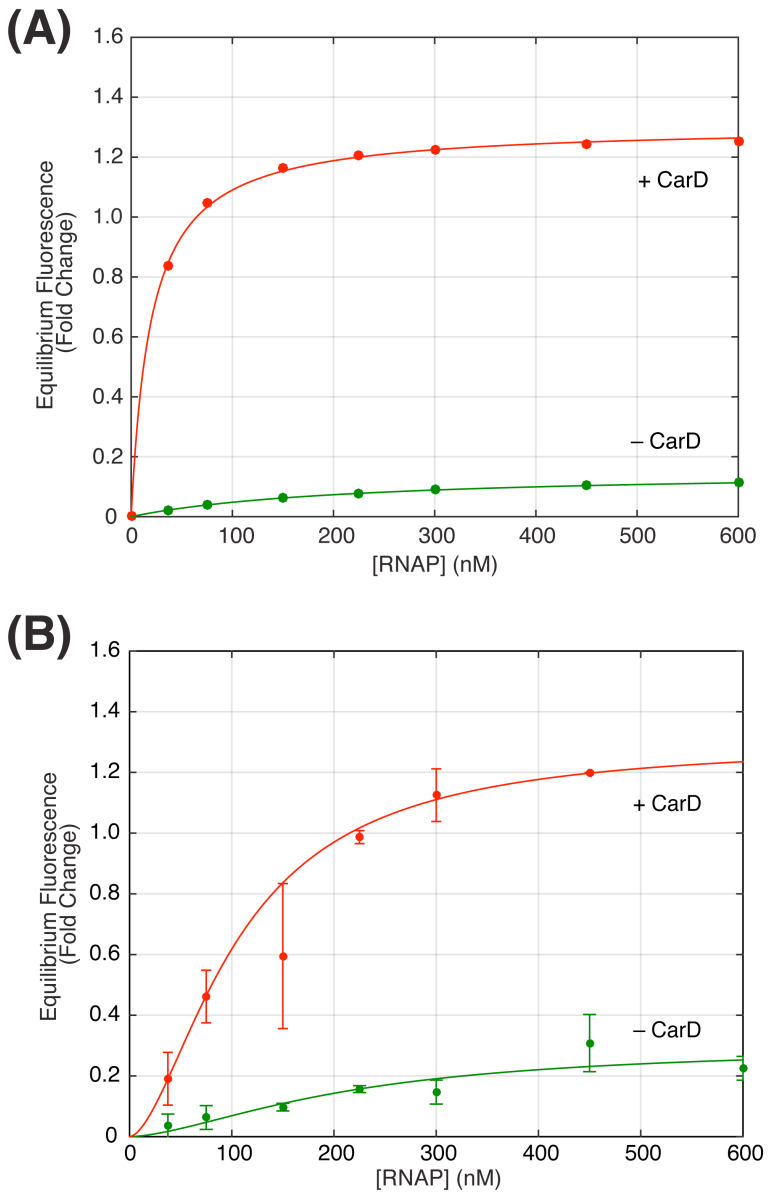


Figure S5: Simulated equilibrium fold enhancements. (A) Using the kinetic model, equilibrium fold changes were simulated and plotted as a function of RNAP concentration in the presence (red) and absence (green) of 1 μM CarD. **(B)** Experimental equilibrium fold changes for the same conditions from the main text (Figure 4B).

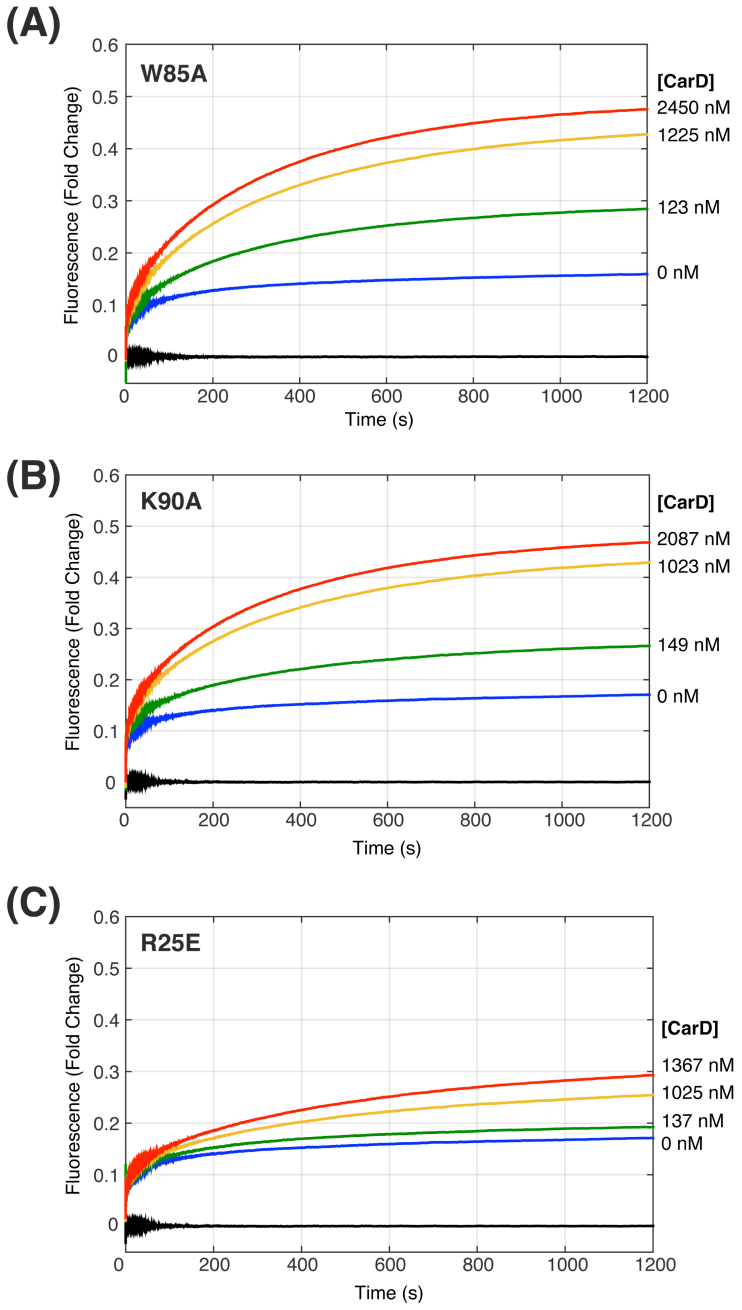


Figure S6: Concentration titrations of mutant CarDs. Fluorescent fold change as a function of time for the **(A)** W85A, **(B)** K90A, and **(C)** R25E mutant CarDs in the presence of 225 nM *Mbo*RNAP.

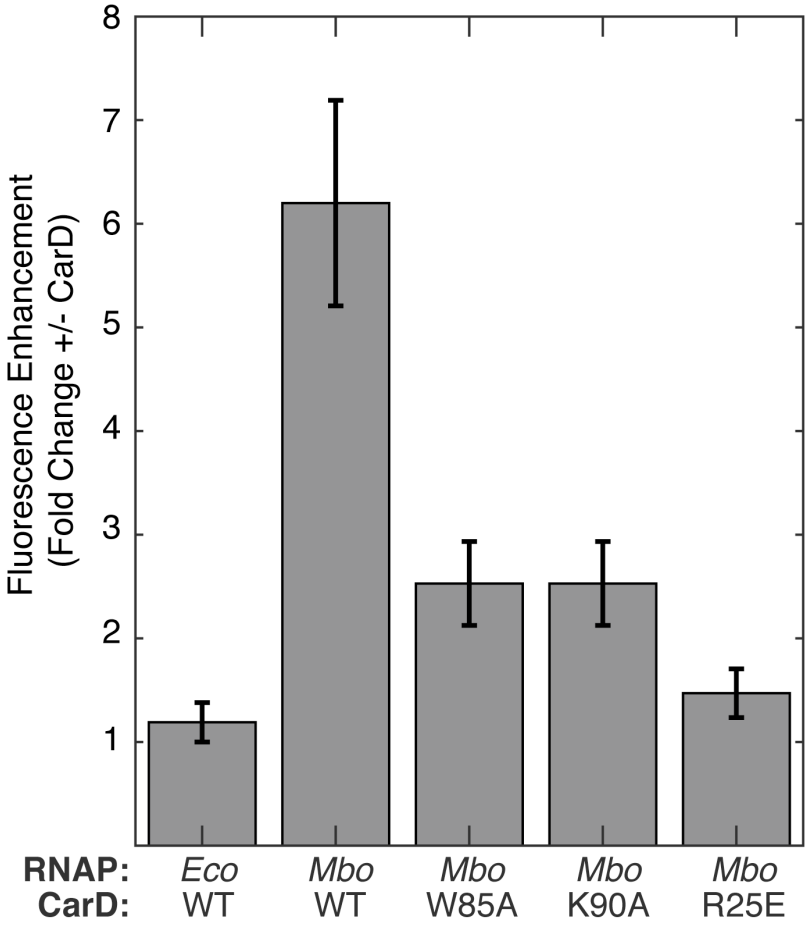


Figure S7: Comparison of mutant CarD-dependent fold-changes. The fluorescent fold changes for 21 nM *Eco*RNAP or 225 nM *Mbo*RNAP with WT or mutant CarD. The largest effect is seen for *Mbo*RNAP and WT CarD. Both W85A and K90A show about half the effect and R25E with *Mbo*RNAP shows an effect almost as low as *Eco*RNAP with WT CarD.