

Supplementary Information

Supplementary Figures

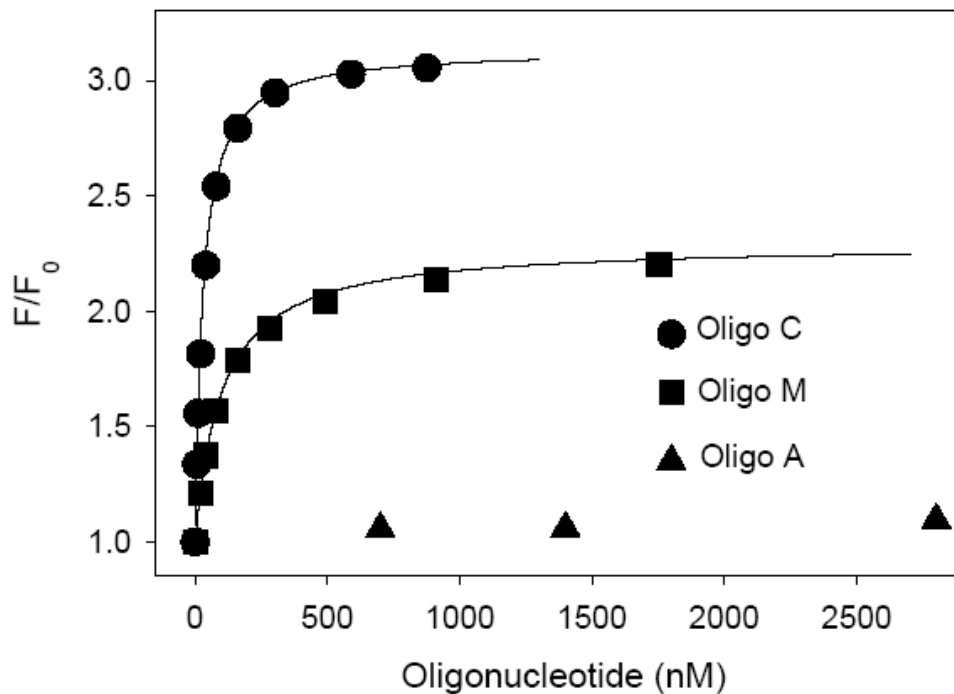
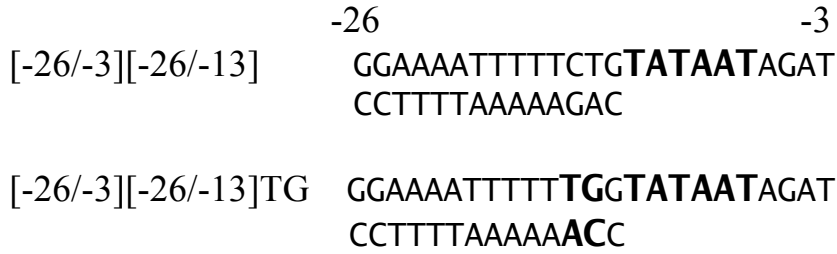


Figure S1. Representative data for the quantification of ss oligo binding to holo RNAP using the beacon assay.

Titration of [211Cys-TMR] σ^{70} holoenzyme RNAP with oligos C, M, A. Sequences of oligos C, M, A are: 5' -ATTGGGTATAATTGACTCA, 5' -ATTGGGCATAATTGACTCA and 5' -ATTGGGATCTCGTGACTCA, respectively, with the -10 element in larger letters.

The solid lines correspond to a nonlinear regression fit of the data to Eq. 1.

A



B

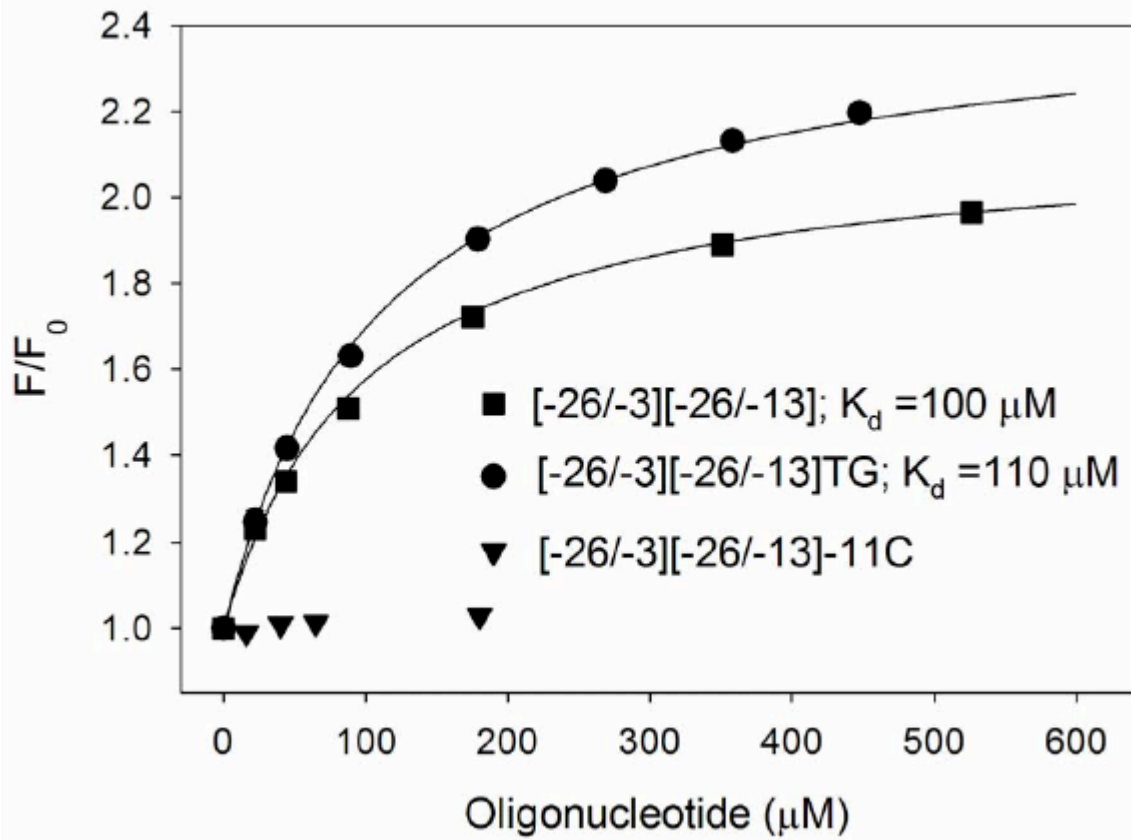


Figure S2. Binding of a fork junction DNA probes to free σ^{70} measured using protein beacon assay. (A) Sequence of fork junction probes with the consensus -10 element and the TG motif sequences highlighted in larger size font. (B) Titration of free [211Cys-TMR] σ^{70} with [-26/-3][-26/-13], [-26/-3][-26/-13]TG and a derivative of [-26/-3][-26/-12] bearing a substitution from A to C at -11. The solid lines correspond to a nonlinear regression fit of the data to Eq. 1.

A

-58 UP element -35 element -14

[-58/-14] GCTAAAATTTTTTTTTTAAAAGTATTTGACATCAGGAAAATTTTTTTG
CGATTTTAAAAAAATTTTCATAAACTGTAGTCCTTTTAAAAAAC

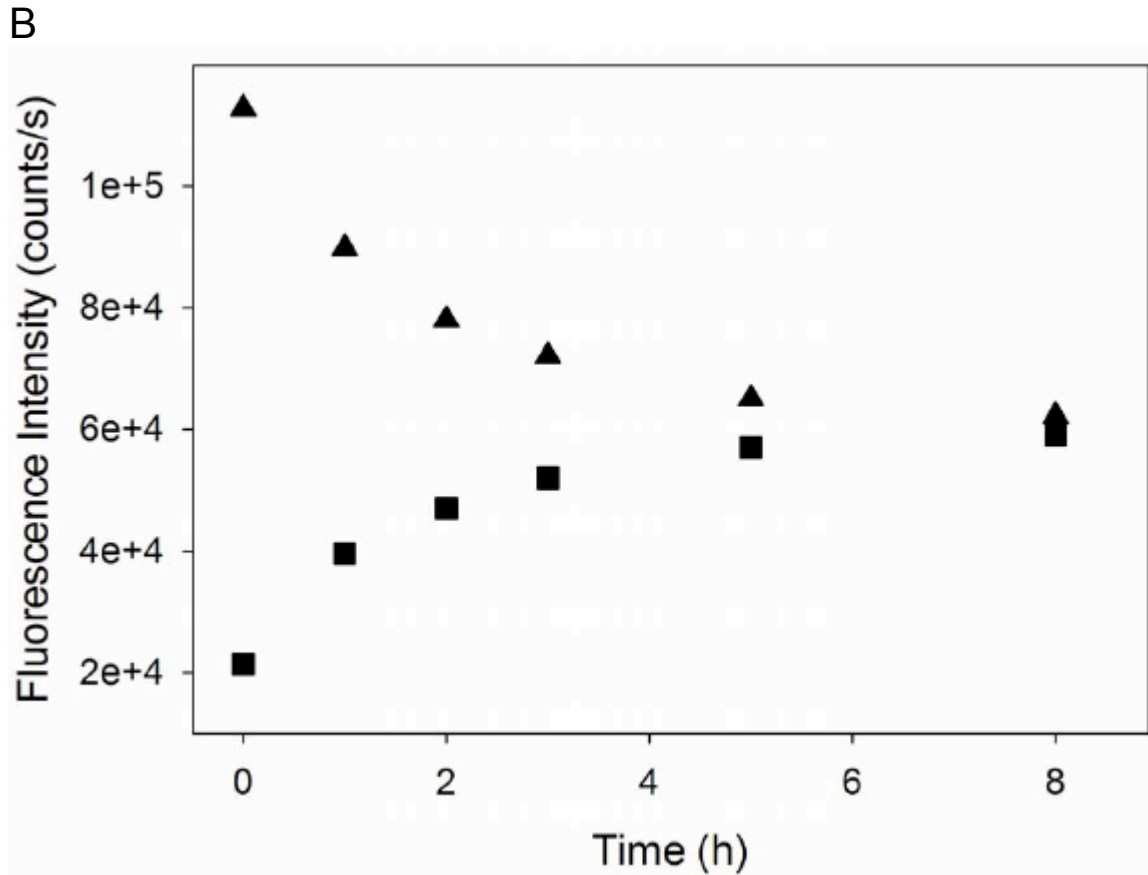


Figure S3. A competition assay measures RNAP binding to fork junction DNA probe. (A) Sequence of a reference competitor probe [-58-14]. (B) An order of addition experiment reveals that competition assay for RNAP binding to [-38/-3][-38/-12];-35mut fork junction reaches equilibrium.

Two samples containing both 1nM [211Cys-TMR] σ^{70} RNAP and either 2nM [-38/-3][-38/-12];-35mut (sample #1) or 4 nM [-58/-14] (sample #2) were incubated for 40 m. Then 4 nM [-58-14] and 2nM [-38/-3][-38/-12];-35mut were added to the samples #1 and

#2, respectively, and fluorescence intensity of the samples was monitored (triangles and squares, respectively).

Supplementary Table

Table S1. Binding of holoRNAP to Oligonucleotides C and M.

RNAP derivative	K_d (C), nM	K_d (M), nM	$K_d(M)/K_d(C)$
RNAP-(σ -211-TMR)	28.8	100	3.5
RNAP-(σ -211-BODIPY FL)	6.2	25.8	4.2
RNAP-(σ -192-TMR)	13.8	55.3	4
^a wt RNAP	3	15	5

The K_d for RNAP beacons are from 2-3 individual experiments, the error is $\pm 15\%$.

^aThe data for wt RNAP are from (Marr, Roberts, Cell 1996).