Supplementary Table 1. Values for the rate and amplitude of each phase in the kinetics of appearance of fractional saturation for the time-resolved X-ray footprinting experiments.

Wild type T7A1 promoter, 37°C, Template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m55–m53	1.15 ± 0.15	0.032 ± 0.006	57.1	42.9
m45–m42	0.98 ± 0.29	0.067 ± 0.018	46.5	53.5
m34–m31	0.99 ± 0.23	0.069 ± 0.014	48.6	51.4
m23–m20	0.82 ± 0.22	0.079 ± 0.014	41.4	58.6
m19–m13	0.31 ± 0.06	0.016 ± 0.006	57.2	42.8
m12–m9	0.67 ± 0.12	0.062 ± 0.010	47.5	52.5
m8–m6	0.36 ± 0.09	0.049 ± 0.013	51.5	48.5
m5–m3	0.17 ± 0.03	0.018 ± 0.012	74.4	25.6
m2–p2	0.34 ± 0.09	0.051 ± 0.011	46.1	53.9
p3–p6	0.16 ± 0.02	0.015 ± 0.005	67.3	32.7
p7–p9	0.16 ± 0.06	0.037 ± 0.023	57.9	42.1
p10–p13	0.18 ± 0.03	0.028 ± 0.015	71.0	29.0
p14–p20	0.20 ± 0.06	0.021 ± 0.006	45.3	54.7

Wild type T7A1 promoter, 37°C, Non–template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m73–m70	1.86 ± 0.25	0.048 ± 0.020	73.5	26.5
m62–m58	1.70 ± 0.29	0.046 ± 0.010	54.6	45.4
m52–m48	1.73 ± 0.24	0.025 ± 0.014	74.2	25.8
m41–m38	0.86 ± 0.10	0.018 ± 0.013	72.7	27.3
m31–m27	0.91 ± 0.22	0.150 ± 0.055	60.4	39.6
m20–m14	0.71 ± 0.07	0.039 ± 0.008	61.3	38.7
m12–m11, m9–m5	0.54 ± 0.06	0.064 ± 0.016	66.6	33.4
m4–p2	0.33 ± 0.05	0.034 ± 0.012	67.0	33.0
p3–p6	0.36 ± 0.04	0.026 ± 0.012	72.4	27.6
p7–p12	0.20 ± 0.03	0.027 ± 0.028	80.4	19.6
p15–p20	0.32 ± 0.08	0.041 ± 0.015	55.5	44.5

Supplementary Table 1A. Values for the apparent rate constants and the amplitudes of both phases as a function of nucleotide position on the template (top panel) and non–template strand (bottom panel) for the case of *E.coli* RNAP holoenzyme–wild type *T7A1* complex formation at 37°C.

Mutant T7A1 promoter (consensus -10 region), 37°C, Template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m55–m53	2.46 ± 0.62	0.183 ± 0.075	60.6	39.4
m45–m42	2.01 ± 0.43	0.260 ± 0.072	56.4	43.6
m33–m31	2.02 ± 0.78	0.301 ± 0.099	45.9	54.1
m23–m20	1.68 ± 0.53	0.239 ± 0.054	43.6	56.4
m18–m14	1.32 ± 0.71	0.239 ± 0.094	43.6	56.4
m13–m10	1.07 ± 0.23	0.151 ± 0.042	56.9	43.1
m9–m6	1.18 ± 0.32	0.196 ± 0.043	46.7	53.3
m5–m3	0.99 ± 0.19	0.138 ± 0.030	52.5	47.5
m2–p1	1.06 ± 0.26	0.165 ± 0.035	47.5	52.5
p2–p4	0.94 ± 0.35	0.179 ± 0.040	39.4	60.6
p5–p9	0.92 ± 0.19	0.163 ± 0.028	47.2	52.8
p10–p13	0.65 ± 0.16	0.166 ± 0.037	48.2	51.8
p14–p20	0.69 ± 0.24	0.187 ± 0.069	51.7	48.3

Mutant T7A1 promoter (consensus -10 region), 37°C, Non–template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m72–m68	2.07 ± 0.29	0.107 ± 0.038	74.0	26.0
m63–m58	1.94 ± 0.55	0.109 ± 0.023	45.8	54.2
m53–m48	1.41 ± 0.39	0.104 ± 0.013	34.1	65.9
m42–m38	1.13 ± 0.38	0.099 ± 0.013	30.9	69.1
m31–m26	0.78 ± 0.24	0.085 ± 0.010	28.5	71.5
m20–m17	0.72 ± 0.14	0.062 ± 0.006	35.2	64.8
m16–m15	0.90 ± 0.33	0.097 ± 0.017	34.1	65.9
m14–m13	0.64 ± 0.31	0.078 ± 0.017	31.7	68.3
m12–m11	0.71 ± 0.19	0.070 ± 0.009	34.4	65.6
m9–m4	0.63 ± 0.13	0.073 ± 0.007	33.2	66.8
m3–p2	0.44 ± 0.08	0.059 ± 0.006	37.5	62.5
p3–p7	0.44 ± 0.09	0.058 ± 0.007	39.4	60.6
p8–p13	0.42 ± 0.11	0.065 ± 0.009	34.8	65.2
p14–p20	0.19 ± 0.04	0.032 ± 0.007	49.0	51.0

Supplementary Table 1B. Values for the apparent rate constants and the amplitudes of both phases as a function of nucleotide position on template (top panel) and non–template strand (bottom panel) for the case of *E.coli* RNAP holoenzyme binding to the *T7A1* promoter mutant with the consensus -10 sequence at 37°C.

Wild type T7A1 promoter, 20°C, Template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m46−m42	0.88 ± 0.12	0.050 ± 0.024	77.7	22.3
m34−m29	1.06 ± 0.18	0.106 ± 0.036	65.0	35.0
m23−m19	0.96 ± 0.11	0.076 ± 0.014	62.1	37.9
m18−m16	0.70 ± 0.18	0.046 ± 0.015	53.7	46.3
m15−m8	0.86 ± 0.09	0.089 ± 0.013	56.1	43.9
m7−m6	0.81 ± 0.19	0.041 ± 0.007	41.0	59.0
m5−m3	0.66 ± 0.18	0.046 ± 0.006	33.3	66.7
m2−p3	1.18 ± 0.30	0.078 ± 0.010	36.7	63.3
p4−p9	0.59 ± 0.11	0.032 ± 0.003	31.2	68.8
p10−p17	0.72 ± 0.17	0.029 ± 0.003	31.0	69.0

Wild type T7A1 promoter, 20°C, Non−template strand

Nucleotide position	Apparent rates (s^{-1})		% of total amplitude	
	k_A	k_B	A	B
m72−m70	2.52 ± 0.39	0.142 ± 0.100	81.9	18.1
m63−m57	2.36 ± 0.31	0.137 ± 0.033	65.8	34.2
m53,m52,m48	1.38 ± 0.23	0.072 ± 0.030	71.4	28.6
m51−m49	1.75 ± 0.22	0.130 ± 0.038	71.3	28.7
m43,m42,m38	1.13 ± 0.30	0.160 ± 0.104	70.8	29.2
m41−m39	1.07 ± 0.14	0.080 ± 0.033	72.6	27.4
m32−m30,m26	0.84 ± 0.14	0.084 ± 0.027	65.3	34.7
m29−m27	0.85 ± 0.12	0.084 ± 0.030	70.7	29.3
m20−m18	0.84 ± 0.17	0.102 ± 0.040	64.5	35.5
m17−m14	0.75 ± 0.11	0.087 ± 0.021	61.6	38.4
m9−m7	0.76 ± 0.24	0.127 ± 0.057	58.2	41.8
m6−m3	0.68 ± 0.16	0.058 ± 0.011	42.1	57.9
m2−p2	0.39 ± 0.13	0.047 ± 0.008	32.3	67.7
p3−p6	0.41 ± 0.11	0.049 ± 0.008	36.0	64.0
p7−p12		0.048 ± 0.002		
p13−p20		0.056 ± 0.003		

Supplementary Table 1C. Values for the apparent rate constants and the amplitudes of both phases as a function of nucleotide position on template (top panel) and non–template strand (bottom panel) for the case of *E.coli* RNAP holoenzyme–wild type *T7A1* complex formation at 20°C.