Supplementary Information

Activity of promoter-bound complexes

In order to determine the fraction of active RP_o among the ensemble of promoter bound complexes, we have counted the number of stalled elongation complexes (SEC) and the number of promoter-bound complexes under conditions of RNA synthesis. Promoter-bound complexes were deemed "inactive promoter complexes" (defined here as IPC) because the associated RNAP was unable to initiate chain elongation in the presence of nucleotides. These experiments were carried out using *wt* RNAP and DNA templates containing either one of the following promoters: λ P_R, P_R-SUB(-463 to -36), *lacUV5*(ICAP), *lacUV5*(UP^{full}) and a 379 bp long G-less cassette downstream of the transcription start site (Supplemental Figure S1). By carrying out transcription in the absence of GTP, an RNA polymerase associated with SEC is at 379 bp (~120 nm) from the promoter and thus it is easily distinguishable from an RNAP that did not move from the promoter. AFM imaging of these complexes (Supplemental Figure S1) allowed us to directly count the number of SEC (originated from active RP_o) and the number of IPC. The resulting data are summarized in Supplemental Table S1, which shows that among the different promoters the fraction of active promoter complexes ranges from 70 to 80 percent. As previously observed (Rivetti *et al*, 2003), the RNA transcript of SEC is not always visible, probably because of RNA degradation or RNA dissociation from the ternary complex.

Beside the determination of the fraction of active RP_o, these experiments make it possible to analyze the conformation of IPC and to compare it with that of the ensemble of promoter bound complexes (RP_o + IPC). Because the DNA fragments used to assemble the complexes have a different length, to facilitate comparison the data are plotted in terms of difference between the mean contour length of bare DNA and the contour length of each complex (Supplemental Figure S2; see Supplemental Table S2 for the complete data set). The data show that in the case of promoters with a large DNA compaction, namely λ P_R and *lacUV5*(UP^{full}), the DNA compaction of IPC is significantly smaller than the DNA compaction of the corresponding RP_o. Conversely, in the case of P_R-SUB(-463 to -36) and *lacUV5*(ICAP) promoters, IPC and RP_o have similar DNA compactions.

DNA templates

The 1054 bp long DNA template λ P_R was obtained by Hind III digestion of plasmid pSAP (Rivetti *et al*, 1999). Even though λ P_R template harbors both P_R and P_{RM} promoters, RNAP prevalently binds to P_R (Fong *et al*, 1993; Hershberger and deHaseth, 1991) as also demonstrated by the outcome of single round transcription experiments (data not shown). The 963 bp long DNA template P_R-SUB(-463 to -36) was obtained by PCR from plasmid pPR35 using primers 5'-AAAACCTCTGACACATGCAGC and 5'-GCTGCCCTTTTGCTCACATG. pPR35 was constructed by cloning the pSAP sequence from -35 to +100 into the HincII restriction site of pNEB193. The 832 bp long DNA template harboring the *lacUV5*(ICAP) promoter was obtained by PCR from plasmid pNEB-lacICAP2 using primers 5'-GAGACGGTCACAGCTTGTCTG and 5'-GGTATCTTTATAGTCCTGTCGGG, Deep Vent DNA polymerase in standard reaction conditions. pNEB-lacICAP2 was obtained by cloning the 249 bp DNA fragment between positions 6032 and 6280 of a M13MP2 derivative into the two PvuII sites of pNEB193 (New England Biolabs). M13MP2 derivative was obtained with the following mutations: 6170-6171 TT, to give a consensus -10 region and 6107-6128 AAATGTGATCTAGATCACATTT to insert the CAP DNA binding site. The 1050 bp long DNA fragment containing the *lacUV5*(UP^{full}) promoter was obtained by PCR from plasmid pUCNN2UPfc using primers 5'-CACATTTCCCCGAAAAGTGCCAC and 5'-GGTATCTTTATAGTCCTGTCGGG, Deep Vent DNA polymerase in standard reaction conditions. Plasmid pUCNN2UPfc is a pUC19 derivative obtained with the following mutations: 515-516 TT, to give a consensus -10 region and 544-566 GTACTTTTCAAAAAAAAATTTCT to insert the consensus UP element. The 1050 bp long DNA template with the *lacUV5*(UP^{prox}) promoter was obtained by PCR from plasmid pUCNN2UPpc using primers 5'-CACATTTCCCCGAAAAGTGCCAC and 5'-GGTATCTTTATAGTCCTGTCGGG, Deep Vent DNA polymerase in standard reaction conditions. Plasmid pUCNN2UPpc is a pUC19 derivative obtained with the following mutations: 515-516 TT and 544-566 GTACTTTTTTTACCACTGCAGTC to insert the consensus proximal subsite of the UP element.

DNA templates harboring a 379 bp G-less cassette were obtained by PCR as follows. The 1222 bp long DNA fragment containing the λP_R promoter was obtained by PCR from plasmid pSAP-Gless using primers 5'-GAGACGGTCACAGCTTGTCTG and 5'-AATACGCAAACCGCCTCTCC. The 1190 bp long DNA fragment containing the P_R-

SUB(-463 to -36) promoter was obtained by PCR from plasmid pPR35-Gless using primers 5'-CAGTCACGACGTTGTAAAACG and 5'-GGTATCTTTATAGTCCTGTC GGG. The 1191 bp long DNA fragment containing the *lacUV5*(ICAP) promoter was obtained by PCR from plasmid pNEB-lacICAP2-Gless using primers 5'-TCACAGCTTGTCTGTAAGCGG and 5'-CAGCGAGTCAGTGAGCGAGG. The 1191 bp long DNA fragment containing the *lacUV5*(UP^{full}) promoter was obtained by PCR from plasmid pUCNN2UPfc-Gless using primers 5'-TCACAGCTTGTCTG TAAGCGG and 5'-CAGCGAGTCAGTGAGCGAGG. PCR reactions were carried out using Deep Vent DNA polymerase under standard conditions.

DNA fragments were purified on 1% (w/v) agarose gel and recovered by electro elution in an Elutrap apparatus (Schleicher & Schuell, Keene NH). The DNA was phenol-chloroform extracted, ethanol precipitated and resuspended in Tris-EDTA buffer (50 mM Tris-HCl pH 7.4, 1 mM EDTA). The concentration of the DNA was determined by absorbance at 260 nm.

Protein purification

Wild-type RNAP was prepared as in (Naryshkin *et al*, 2001; Tang *et al*, 1996) and was further purified by Mono-Q chromatography as in (Mukhopadhyay *et al*, 2003), or, alternatively, was prepared as in (Niu *et al*, 1996). $\Delta\alpha CTD^{I}/\Delta\alpha CTD^{II}$ RNAP, $\Delta 6\text{-}\alpha^{I}/\Delta 6\text{-}\alpha^{II}$ RNAP, and $\Delta 12\text{-}\alpha^{I}/\Delta 12\text{-}\alpha^{II}$ RNAP were prepared as in (Naryshkin *et al*, 2001; Tang *et al*, 1996) --using pHTT7f1NHα (Tang *et al*, 1995) derivatives encoding, respectively, N-terminally hexahistidine-tagged α(1-235) (Tang *et al*, 1995), N-terminally hexahistidine-tagged [Δ(236–241)]α (Meng *et al*, 2000), and N-terminally hexahistidine-tagged [Δ(236-247)]α (Meng *et al*, 2000)-- and were further purified by Mono-Q chromatography as in (Mukhopadhyay *et al*, 2003). $\Delta\alpha CTD^{II}$ RNAP was prepared as in (Estrem *et al*, 1999).

Preparation of stalled elongation complexes

Stalled elongation complexes were prepared by first assembling RP_o as described in Methods, followed by addition of 16 U RNAsin (Promega) and a mixture of ATP, CTP and UTP to a final concentration of 100 μM each. The 10 μl reaction was incubated for 15 minutes at 37 °C. Sample deposition and AFM imaging were performed as described in Methods.

Data analysis

DNA molecules and nucleoprotein complexes suited for analysis were selected by visual inspection based on the following criteria: The molecule had to be completely visible in the image, its contour was not ambiguous, the RNAP was bound at the expected position, no other proteins were bound to the same DNA. When possible, the DNA arm ratio was used to further select promoter-bound complexes. Data were elaborated with Matlab and plotted with Sigmaplot. In all graphs, the number of bins was chosen to be the square root of the sample size. Single peak distribution were fitted with a Gaussian function whereas double peak distributions were fitted with a function obtained by the sum of two Gaussian.

Supplemental References

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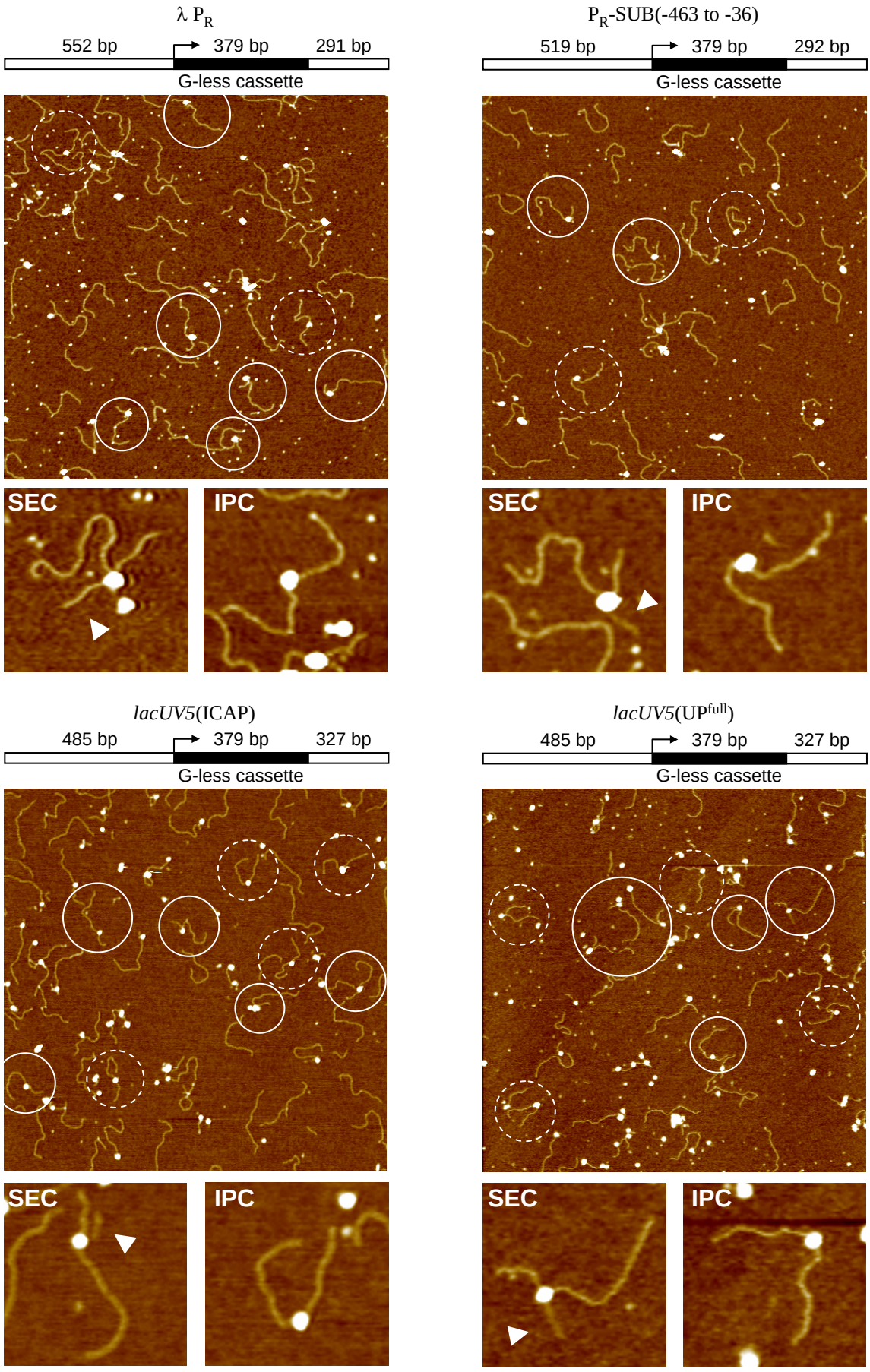
Supplemental Table S1. Number of complexes after addition of ATP, CTP and UTP.

Promoter	Stalled elongation complexes (SEC)	Inactive promoter complexes (IPC)	Active promoter complexes
λ P_R	147	67	69%
P_R-SUB(-463 to -36)	136	40	77%
lacUV5(ICAP)	141	59	70%
lacUV5(UP^{full})	117	36	76%

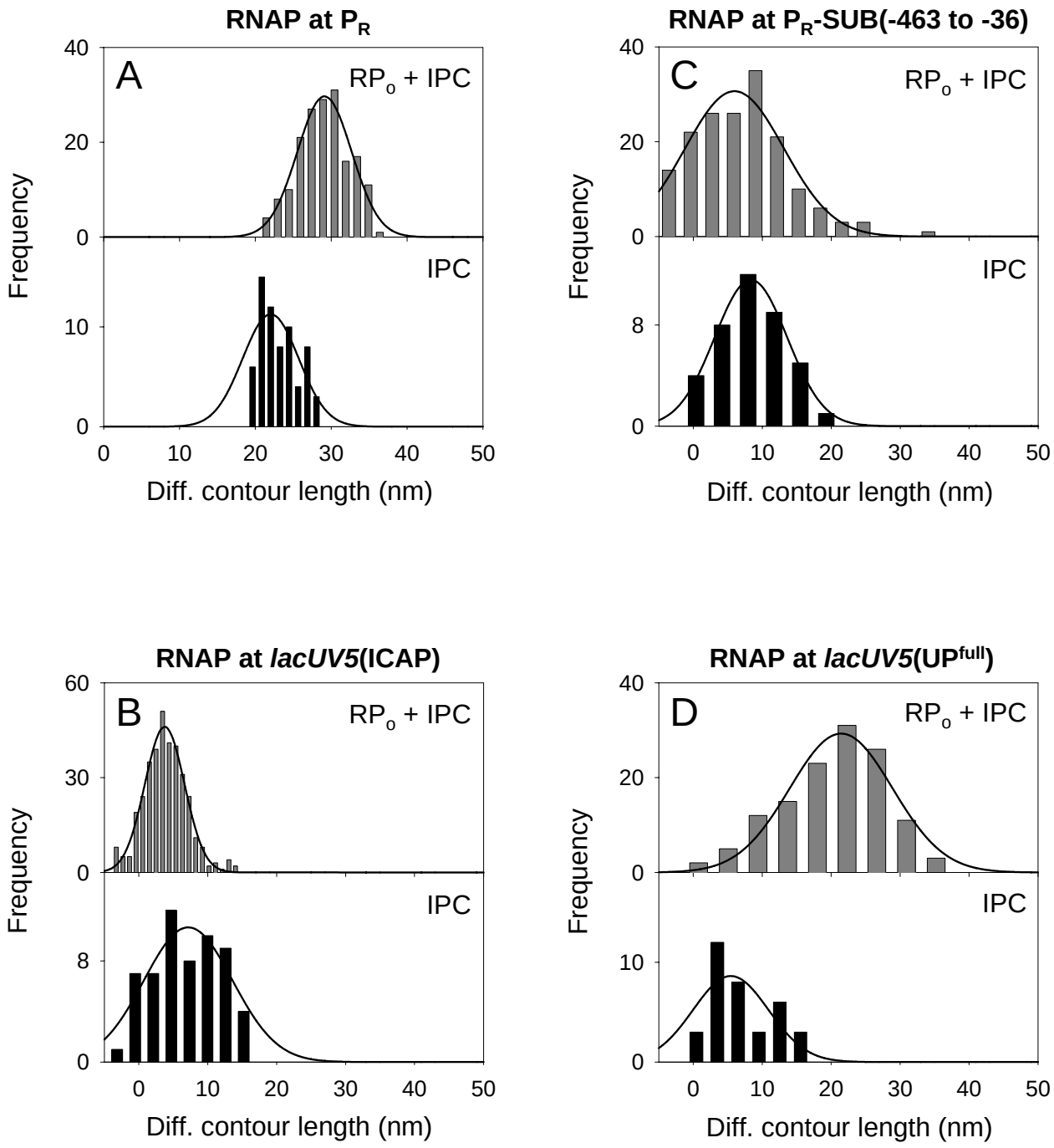
Supplemental Table S2. DNA compaction of inactive promoter complexes.

Promoter	DNA length (bp)	Bare DNA contour length (nm)	DNA contour length of IPC (nm)	DNA compaction (nm)
λ P_R	1222	400 ± 0.5 (N = 81)	378 ± 0.4 (N = 67)	22 ± 0.7
P_R-SUB(-463 to -36)	1190	379 ± 0.4 (N = 57)	371 ± 0.8 (N = 40)	8 ± 0.8
lacUV5(ICAP)	1191	378 ± 0.4 (N = 58)	371 ± 0.8 (N = 59)	7 ± 0.9
lacUV5(UP^{full})	1191	377 ± 0.5 (N = 36)	372 ± 0.9 (N = 36)	5 ± 1.0

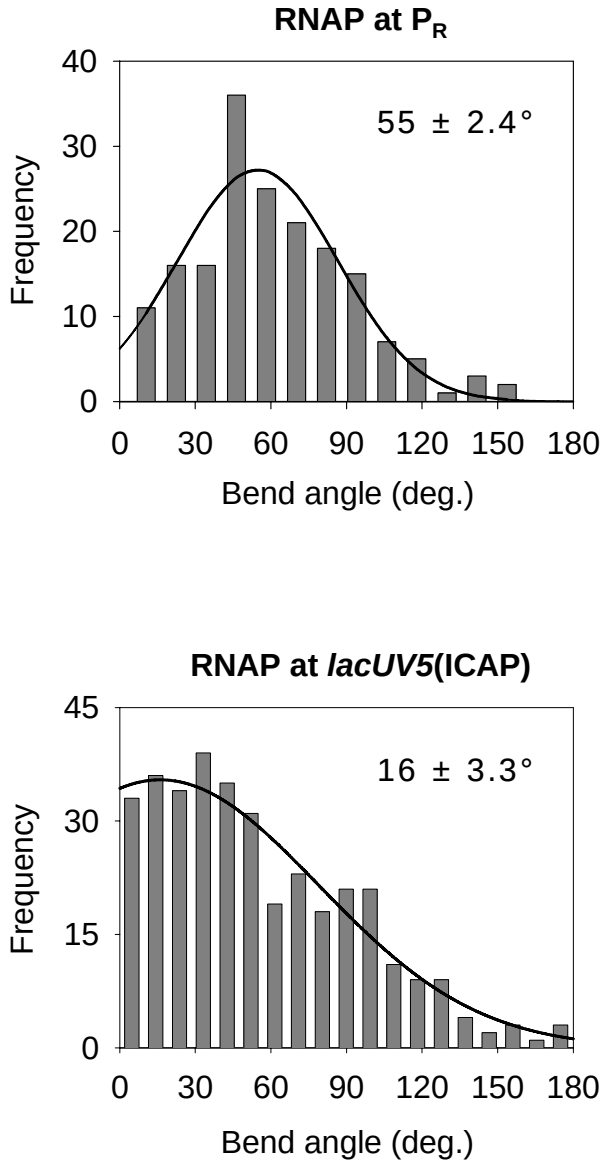
Contour length values of bare DNA and IPC represent the mean ± standard error of the mean (SE) obtained from the fitting of the DNA contour length distributions shown in Supplemental Figure S2. N represents the number of molecules measured for each data set. The DNA compaction is given by the difference between the mean DNA contour length of bare DNA and that of IPC.



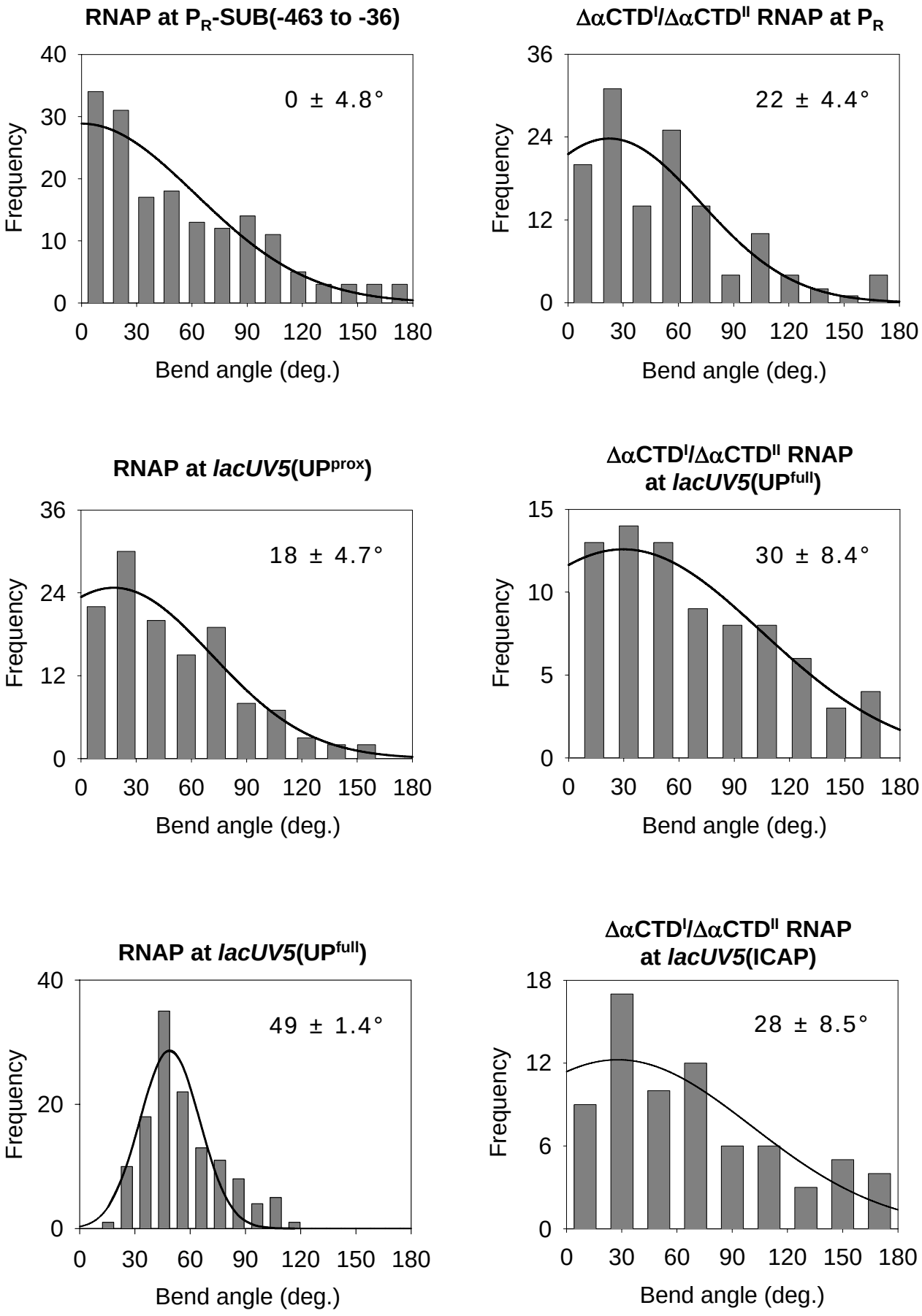
Supplemental Figure S1. AFM images of stalled elongation complexes (SEC) and inactive promoter complexes (IPC). A) λ P_R; B) P_R-SUB(-463 to -36); C) *lacUV5*(ICAP); D) *lacUV5*(UP^{full}). Solid circles highlight SEC, dashed circles highlight IPC. Arrow heads point to RNA transcript. A schematic rapresentation of the DNA template used in the experiments is shown above the corresponding panel. The scan size of the large views is 2 μm whereas the scan size of zoomed images is 293 nm.



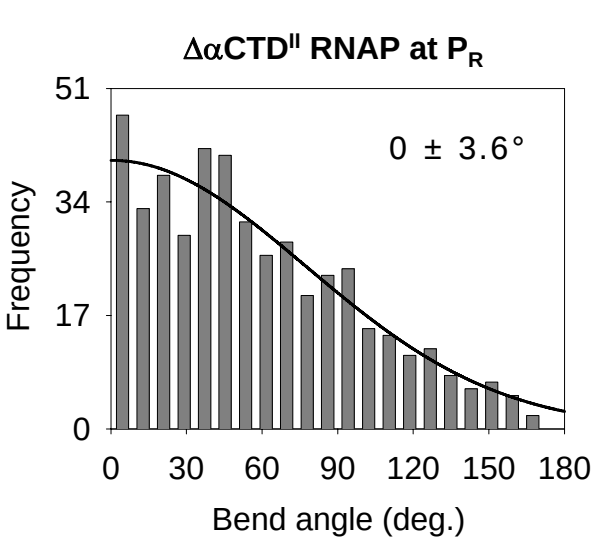
Supplemental Figure S2. DNA compaction of inactive promoter complexes. A) Top graph: distribution of DNA compactions of the promoter complexes shown in Figure 2A; bottom graph: distribution of DNA compactions of inactive promoter complexes at λP_R. B) Top graph: distribution of DNA compactions of the promoter complexes shown in Figure 2B; bottom graph: distribution of DNA compactions of inactive promoter complexes at *lacUV5*(ICAP). C) Top graph: distribution of DNA compactions of the promoter complexes shown in Figure 3A; bottom graph: distribution of DNA compactions of inactive promoter complexes at P_R-SUB(-463 to -36). D) Top graph: distribution of DNA compactions of the promoter complexes shown in Figure 3C; bottom graph: distribution of DNA compactions of inactive promoter complexes at *lacUV5*(UP^{full}). The distribution of DNA compaction was obtained by subtracting the DNA contour length of each RP_o from the mean DNA contour length of protein-free molecules (Table 1 and Supplemental Table S2).

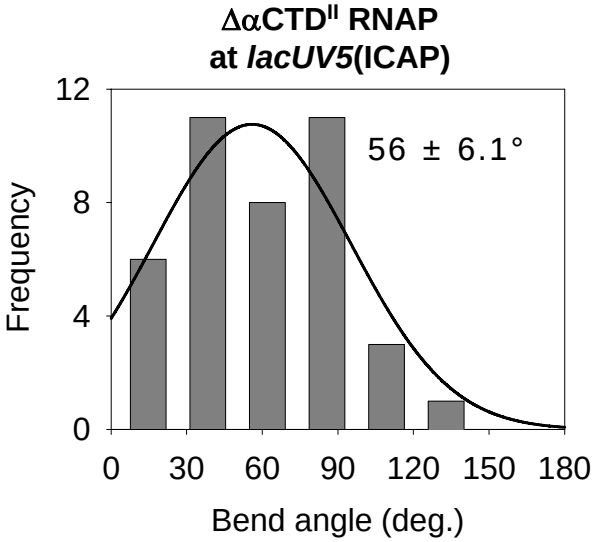


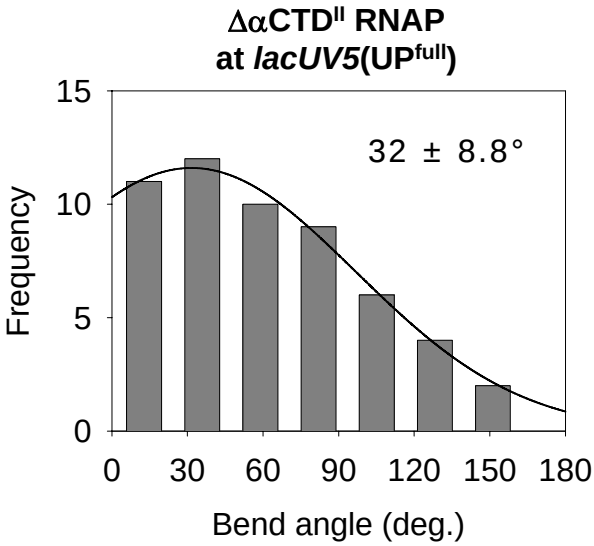
Supplemental Figure S3. DNA bend angle distributions for complexes in Figure 2. The mean DNA bend angle obtained from a Gaussian fitting ±SE is shown in each graph.



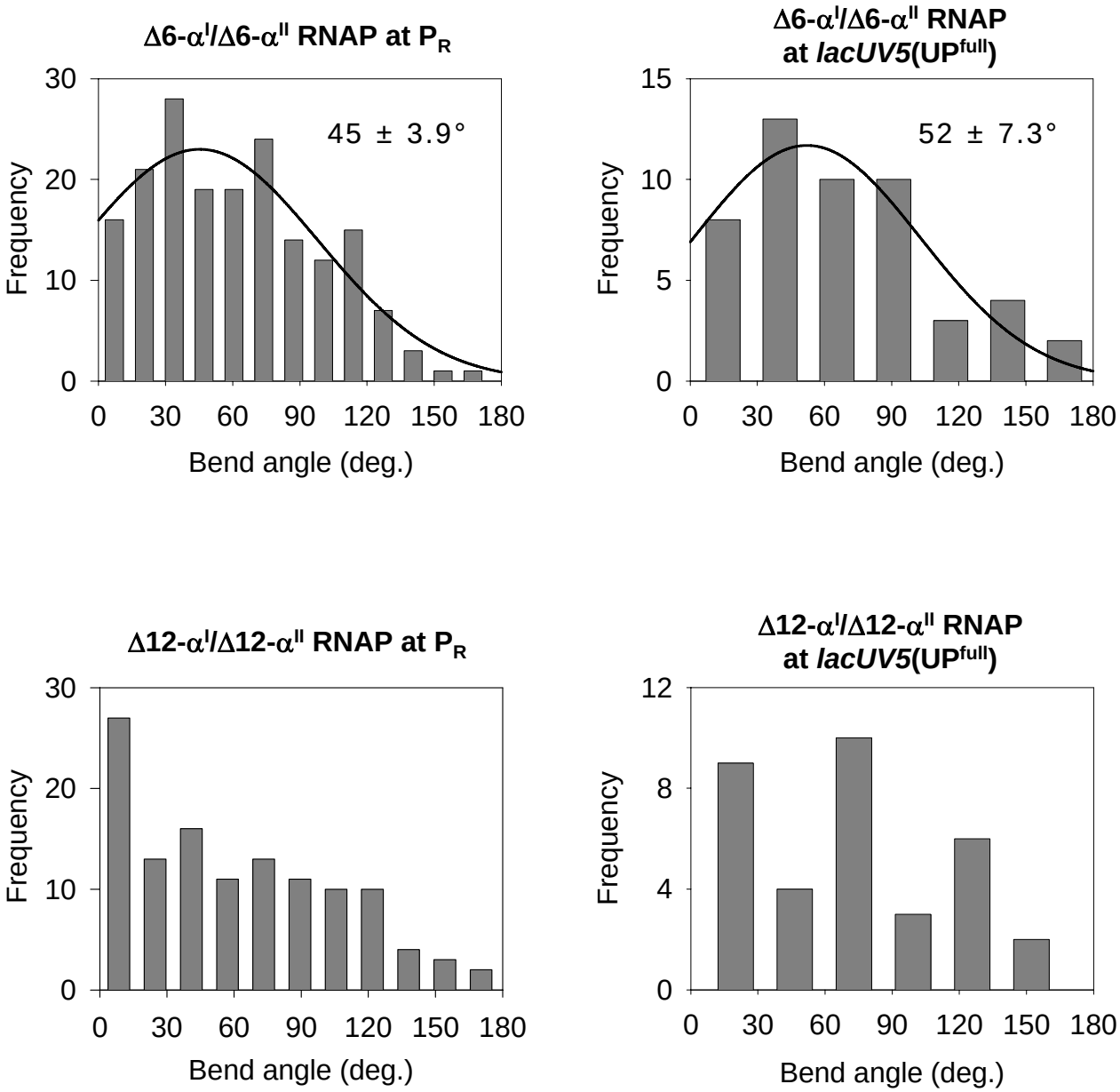
Supplemental Figure S4. DNA bend angle distributions for complexes in Figure 3. The mean DNA compaction ±SE obtained from the Gaussian fitting of the distributions is shown in each graph.







Supplemental Figure S5. DNA bend angle distributions for complexes in Figure 4. The mean DNA compaction ±SE obtained from the Gaussian fitting of the distributions is shown in each graph.



Supplemental Figure S6. DNA bend angle distributions for complexes in Figure 5. The mean DNA compaction ±SE is shown only for those distribution that can be fitted by a Gaussian.