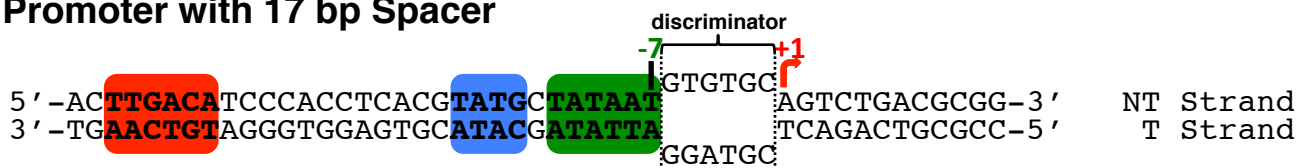


**A**

**DNA Promoter with 17 bp Spacer**



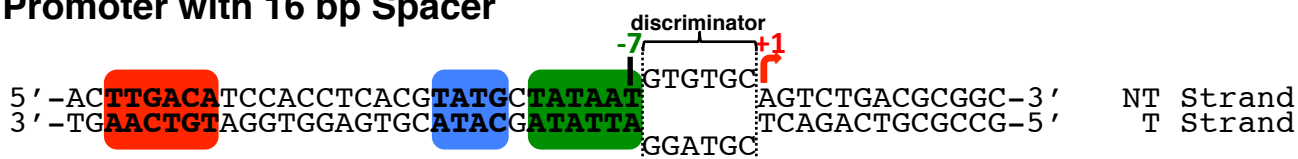
↓ + RNAP  
+ ATP, GTP, UTP

**TIC bubble with 4 nt RNA (TIC1)**



**B**

**DNA Promoter with 16 bp Spacer**



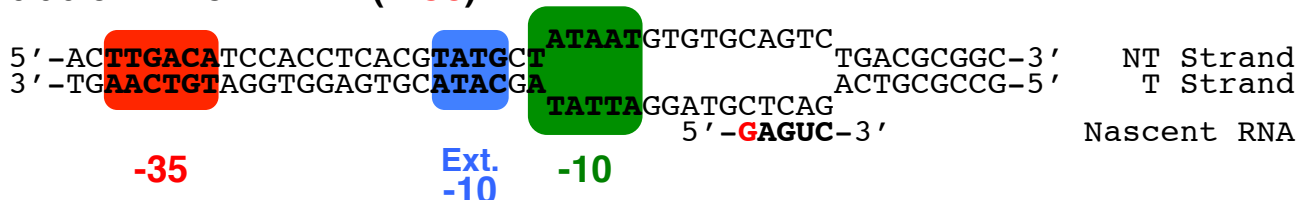
↓ + RNAP  
+ ATP, GTP, UTP

**TIC Bubble with 4 nt RNA (TIC2)**

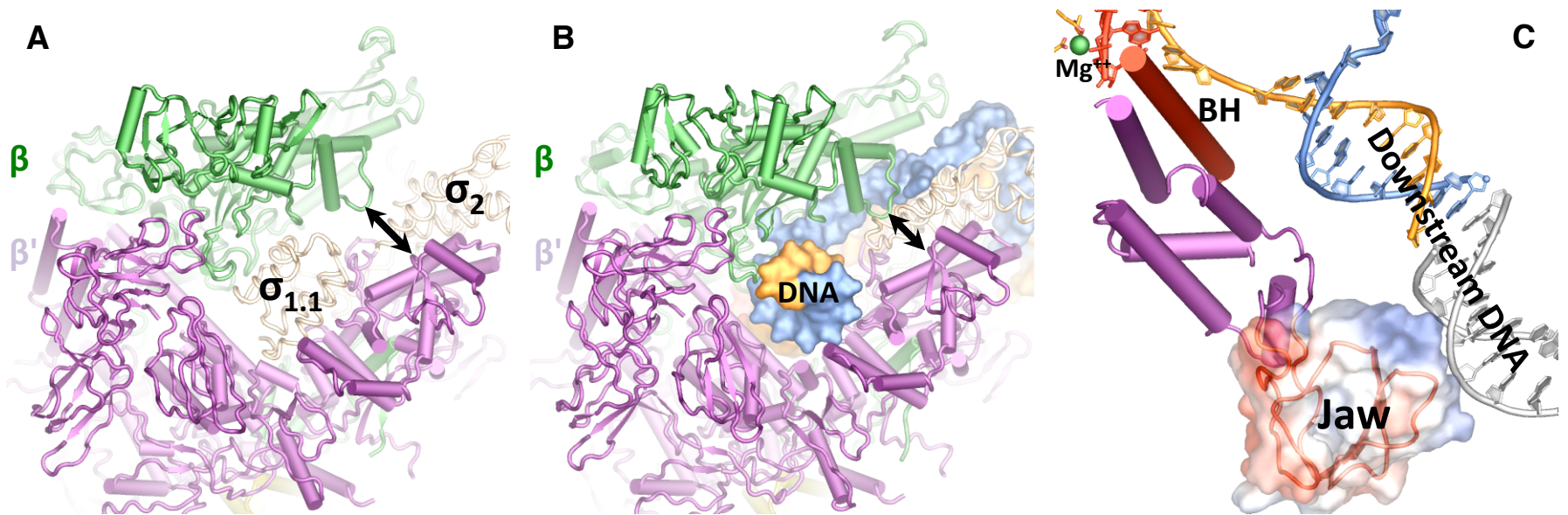


↓ + CTP

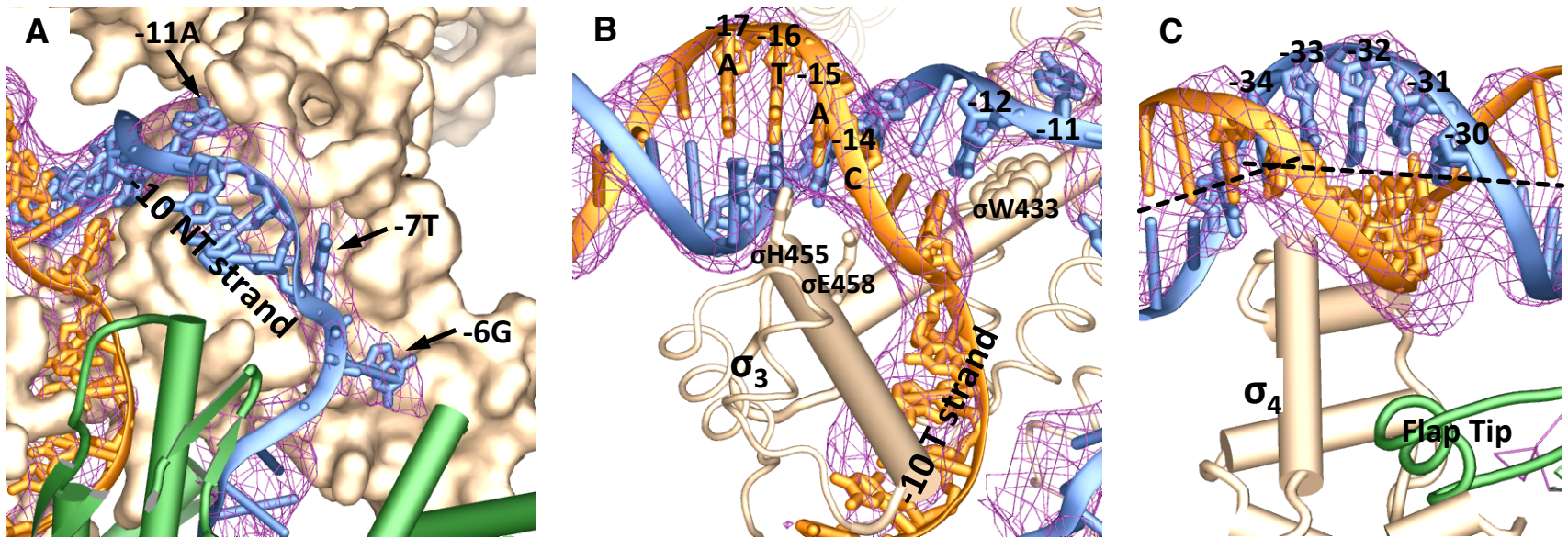
**TIC Bubble with 5 nt RNA (TIC3)**



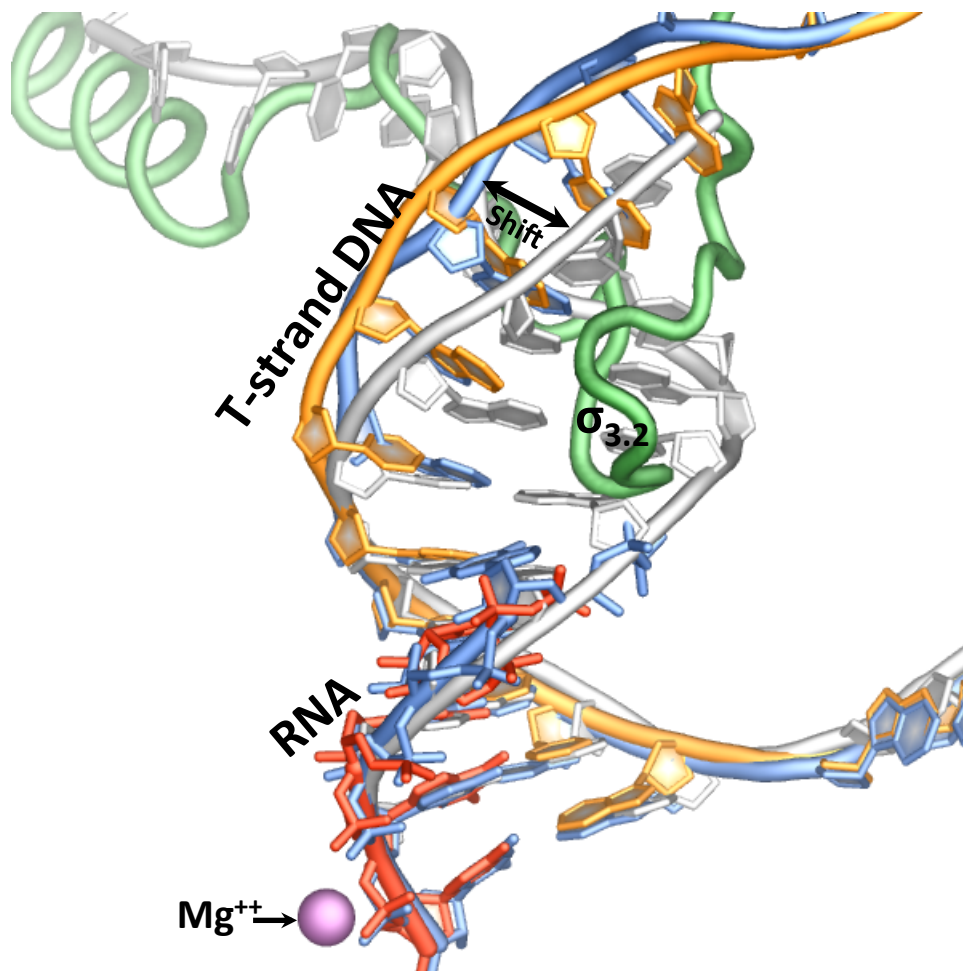
**Figure S1, related to Table 1. Synthetic DNA promoters used for crystallizing transcription initiation complexes.** (A) A 49-bp DNA promoter with a 17-bp spacer. (B) A 49-bp DNA promoter with a 16-bp spacer. Both promoters produce an RNA tetranucleotide from ATP, GTP and UTP. Soaking TIC2 crystals with CTP extends the RNA to 5 nt (TIC3). The conserved -35, -10 and extended -10 sequences were shaded with colors.



**Figure S2, related to Figure 1. The Downstream Primary Channel.** (A) Downstream channel of the *E. coli* RNAP apo holoenzyme. The  $\sigma_{1.1}$  domain in the RNAP apo holoenzyme is modeled based on that reported in literature (Bae et al., 2013). (B) Downstream channel of the transcription initiation complex. The RNAP subunits and nucleic acid strands in both (A) and (B) are color coded as in Fig. 1. (C) A close-up view of the interaction between the jaw domain and the downstream DNA in the TIC. A fraction of the *E. coli* RNAP core, including the catalytic metal center ( $Mg^{++}$ ) and the bridge helix (BH), is shown for reference. The jaw domain is also shown in a surface potential diagram.



**Figure S3, related to Figure 2. Promoter Recognition by  $\sigma^{70}$ .** (A)  $\sigma_2$  interacts with the -10 NT-strand.  $\sigma^{70}$  is shown in a surface representation. (B) Recognition of the extended -10. Two perpendicular  $\alpha$  helices spanning the  $\sigma_2$  and  $\sigma_3$  domains are inserted into the major groove of the extended -10 region. (C)  $\sigma_4$  interacts with the promoter -35 region. DNA strands and protein subunits are colored as in Figure 1. DNA strands are shown in ladders with the conserved -35, -10 and extended -10 residues also shown in sticks. Dashed lines mark the DNA axis that bends toward the protein due to protein-DNA interactions. Purple meshes show the  $\sigma_A$ -weighted  $F_{\text{obs}} - F_{\text{calc}}$  electron density map contoured at  $2.0 \sigma$  for the promoter DNA.



**Figure S4, related to Figure 3. Comparison of the T-strand DNA between the TIC and TEC.** The template DNA strands and the nascent RNA oligonucleotides from the TICs (TIC1, orange and red respectively; TIC3, both strands in blue), and the T-strand DNA and RNA from the transcription elongation complex (TEC, both strands in grey) (Vassilyev et al., 2007) are superimposed using their DNA-RNA hybrids portion backbones. A  $\sigma^{70}$  fragment (green) and the catalytic metal center ( $Mg^{++}$ , purple ball) from TIC1 are shown for reference.