

Supplementary Tables

Supplementary Table 1. The statistics of the cryo-EM structure of *B. subtilis* BmrR-TAC.

	<i>B. subtilis</i> BmrR-TAC (EMDB-30390) (PDB 7CKQ)
Data collection and processing	
Magnification	22,500
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	60.8
Defocus range (μm)	-2.2 to -1.2
Pixel size (Å)	1.0
Symmetry imposed	C1
Initial particle images (no.)	494,295
Final particle images (no.)	103,226
Map resolution (Å)	4.4
FSC threshold	0.143
Map resolution range (Å)	4.0-7.0
Refinement	
Initial model used (PDB code)	6LDI, 3Q3D, 4NJC
Model resolution (Å)	4.4
FSC threshold	0.143
Model resolution range (Å)	4.0-7.0
Map sharpening <i>B</i> factor (Å ²)	-141.5
Model composition	
Non-hydrogen atoms	27,470
Protein residues	3,586
Ligands	5
<i>B</i> factors (Å ²)	
Protein	96.04
Ligand	158.30
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.779
Validation	
MolProbity score	1.91
Clashscore	9.00
Poor rotamers (%)	0.67
Ramachandran plot	
Favored (%)	93.50
Allowed (%)	6.50
Disallowed (%)	0.00

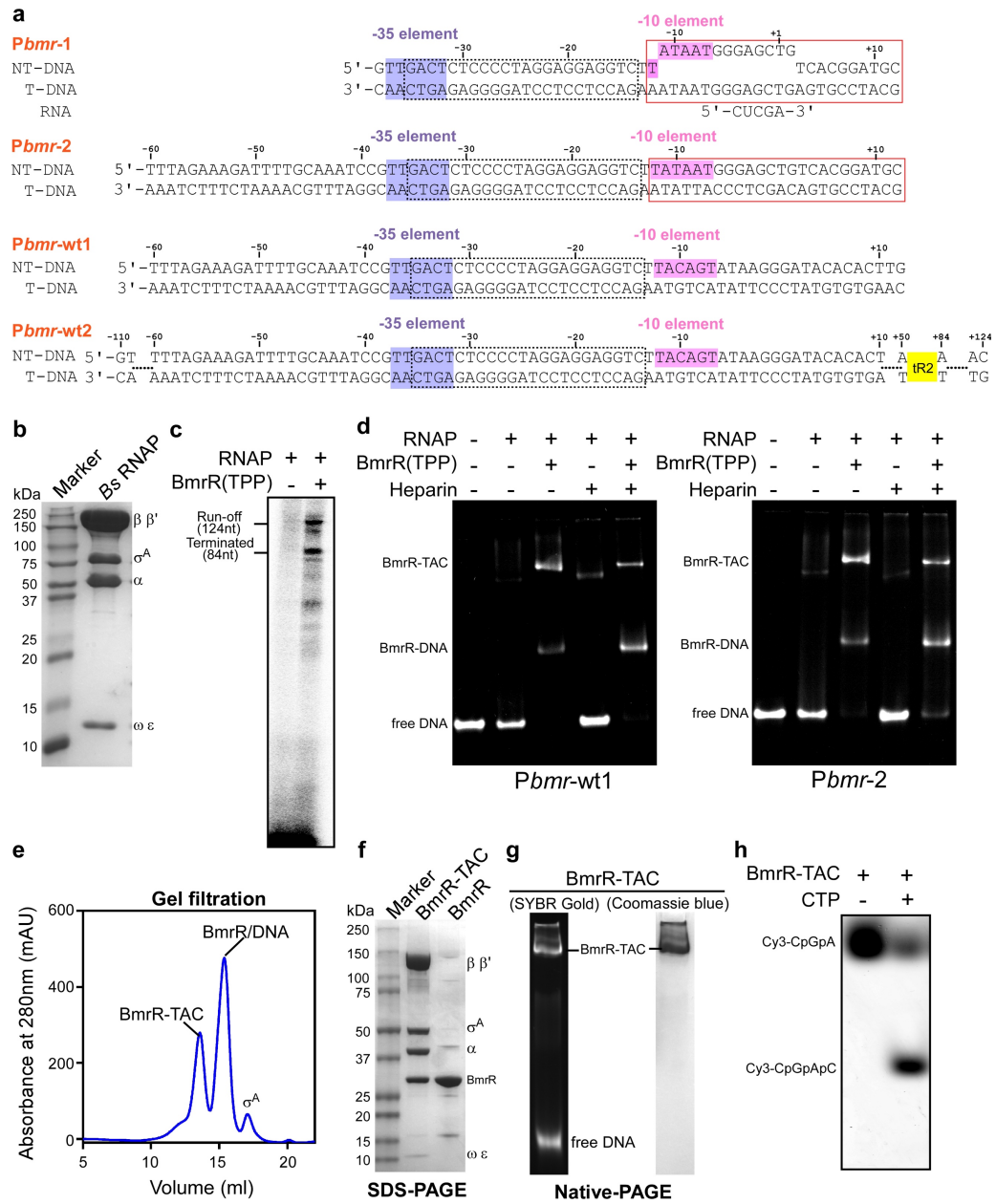
Supplementary Table 2. The sequences of reported MerR-TF regulated promoters with the -35 and -10 elements underlined.

Name	Promoter sequences	Spacer (bp)	Ref.
<i>Tn501/Tn21 Pmer</i> (MerR)	<u>TTGACTCCGTACATGAGTACGGAAGTAAGGT</u>	19	1
<i>B. subtilis Pmer</i> (MerR)	<u>TTTACCCTGTACTAAGGTACGTGGTTTATGCT</u>	20	2
<i>S. aureus Pmer</i> (MerR)	<u>TTGACCGTGTACTATGGTACAGGGTTTATACT</u>	20	3
<i>E. coli PzntA</i> (ZntR)	<u>TTGACTCTGGAGTCGACTCCAGAGTGTATCCT</u>	20	4
<i>E. coli PcopA</i> (CueR)	<u>TTGACCTTCCCCTTGCTGGAAGGTTTAAACCT</u>	19	5
<i>E. coli PcueO</i> (CueR)	<u>TTGACCTTCCCGTAAGGGGAAGGACTATGCT</u>	19	5
<i>Synechocystis PcoaT</i> (CoaR)	<u>TTGACATTGACACTAATGTTAAGGTTTAGGCT</u>	20	6
<i>R. metallidurans PpbrA</i> (PbrR)	<u>TTGACTCTATAGTAACTAGAGGGTGTTAAAT</u>	19	7
<i>P. putida PcadA</i> (CadR)	<u>TTGACCCTATAGTGGCTACAGGGTGTTCACTT</u>	20	8
<i>E. coli PsoxS</i> (SoxR)	<u>TTTACCCTCAAGTTAACTTGAGGAATTATACT</u>	19	9
<i>S. lividans PtipA</i> (TipA _L)	<u>TTGCACCTCACGTCACGTGAGGAGGCAGCGT</u>	19	10
<i>B. subtilis Pbmr</i> (BmrR)	<u>TTGACTCTCCCTAGGAGGAGGTCTTACAGT</u>	19	11
<i>B. subtilis Pblt</i> (BltR)	<u>TTGACTATACGGTAACCATATACCTTATGAT</u>	19	12
<i>B. subtilis Pmta</i> (Mta)	<u>TTGACCCTAACGTTGCGTGATTGTTTACGAT</u>	19	13
<i>P. aeruginosa PbrlR</i> (BrlR)	<u>TTGACCTTGCCCCAGGGGCAATCCGTAGTCT</u>	19	14
<i>N. gonorrhoeae PnmlR</i> (NmlR)	<u>TTGCGCTTAGAGTGAACCTCTAAAATGTAAACT</u>	19	15
<i>R. sphaeroides PcopA</i> (CueR)	<u>TTGACCTTCCAGTTGTGGGAAGCCCCATCTT</u>	19	16
<i>H. influenzae Pnik</i> (NimR)	<u>TTGATTCTAAAGTTACTTCATATTTTATCAT</u>	19	17
<i>B. subtilis PadhR</i> (AdhR)	<u>TTGACTTAAAGTTAACTTTAAGTGTTACCTT</u>	19	18
<i>B. pertussis Pbor</i> (ZccR)	<u>TTGACCCTATAGTAACTCCAGGGTGTTGAAAT</u>	19	19
<i>S. enterica PcuiD</i> (SctR)	<u>TTGACCTTCCCGTTAGGGCAGGGTCTAAGCT</u>	19	20
<i>B. japonicum PnolA</i> (NolA)	<u>TTGAATCATACGTGACGTCAGGTTGTAGGCT</u>	19	21
<i>S. enterica PcopA</i> (CueR)	<u>TTGACCTTAAACCTTGCTGGAAGGTTTAAACCT</u>	19	22
<i>S. enterica PcueO</i> (CueR)	<u>TTGACCTTCCCGTTAGGGCAGGGTCTAAGCT</u>	19	22
<i>S. enterica PcueP</i> (CueR)	<u>TTGACCTTTCCCTTAGGGGAACCCCTATAGT</u>	19	22
<i>S. enterica PgolB</i> (GolS)	<u>TTGACCTTCCAACACTGGCAAGGTCCAGACT</u>	19	22
<i>S. enterica PgolTS</i> (GolS)	<u>TTGACCTTCCCACAATGGCAAGCTTTAGGCT</u>	19	22
<i>S. enterica PgesABC</i> (GolS)	<u>TTGACCTTTCCTTCGTTGTAACGCCTAGCCT</u>	19	22

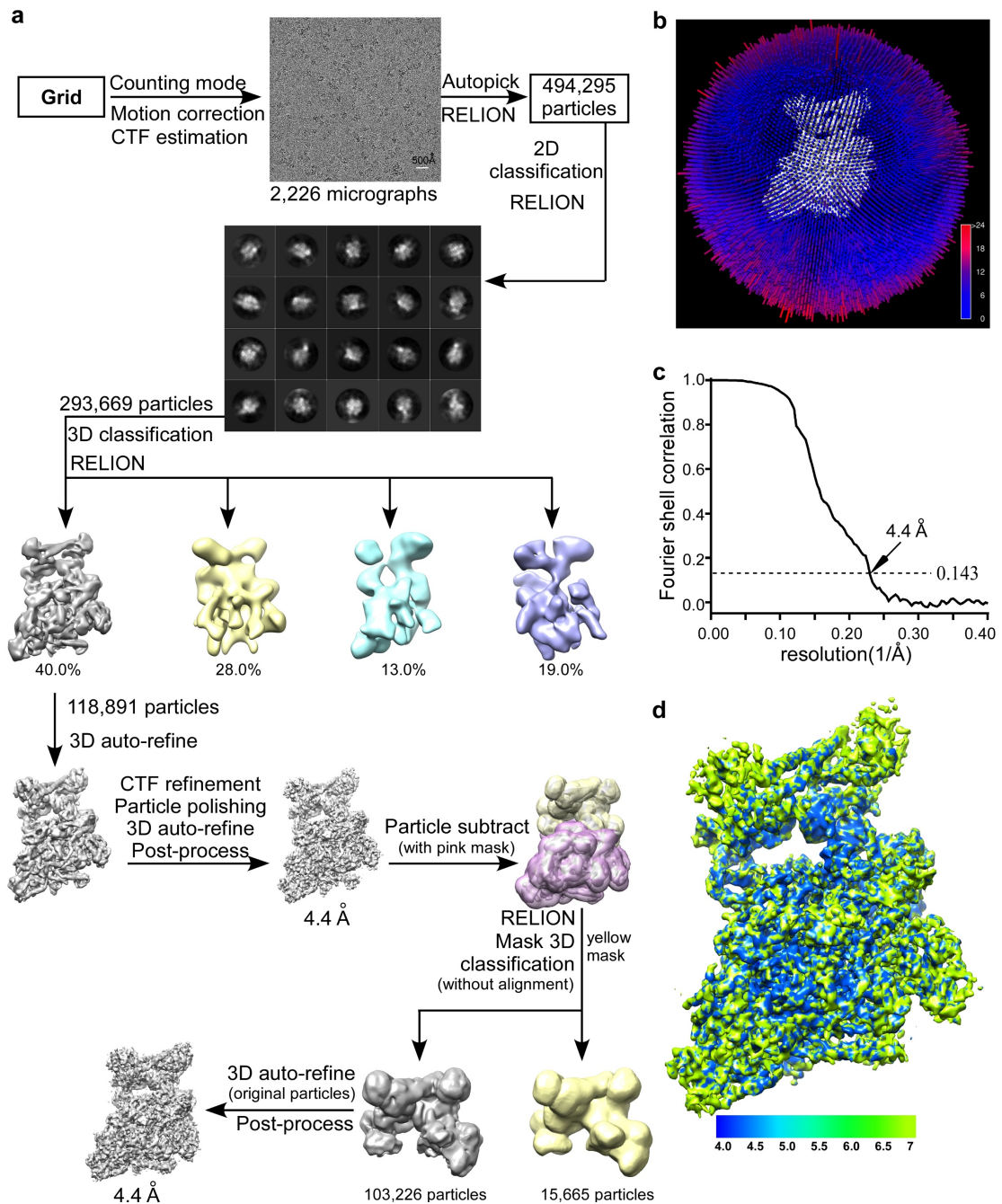
Supplementary Table 3. Oligonucleotides with corresponding sequences (5' to 3') used for plasmid construction and promoter amplify.

Oligonucleotides	Sequences
pTolo-EX5 / <i>Bs</i> BmrR	TATTTTCAGGGCGCCATGGCCATGAAGGAATCGTATTACTCAATTGGGGTGG TGGTGGTGGTGGTGCTCGAGTTATTCAGCGATCCGTATTTTCATTTCGAC
pET28a / <i>Bs</i> σ^A	TATTTTCAGGGCGCCATGGCCATGGCTGATAAAACAAACCCACG TGGTGGTGGTGGTGCTCGAGTTATTCAAGGAAATCTTTCAAACGTTTACTTC TG
pEASY / <i>P-bmr</i>	TTTGCAAATCCGTTGACTCTCCCC AAATAAAAAGGCCTGCGATTACCAGCAGGCCTTAAGGTAATATTTTCTTCT CCATATGAC
M13F:	GTAAAACGACGGCCAGT
M13R:	CAGGAAACAGCTATGAC

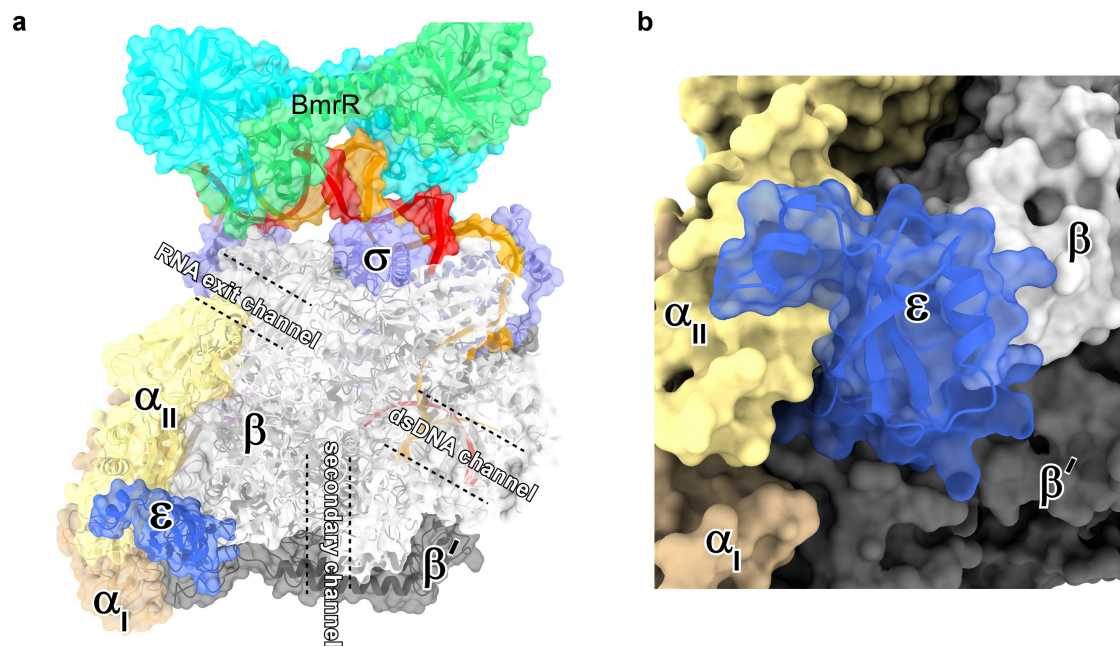
Supplementary Figures



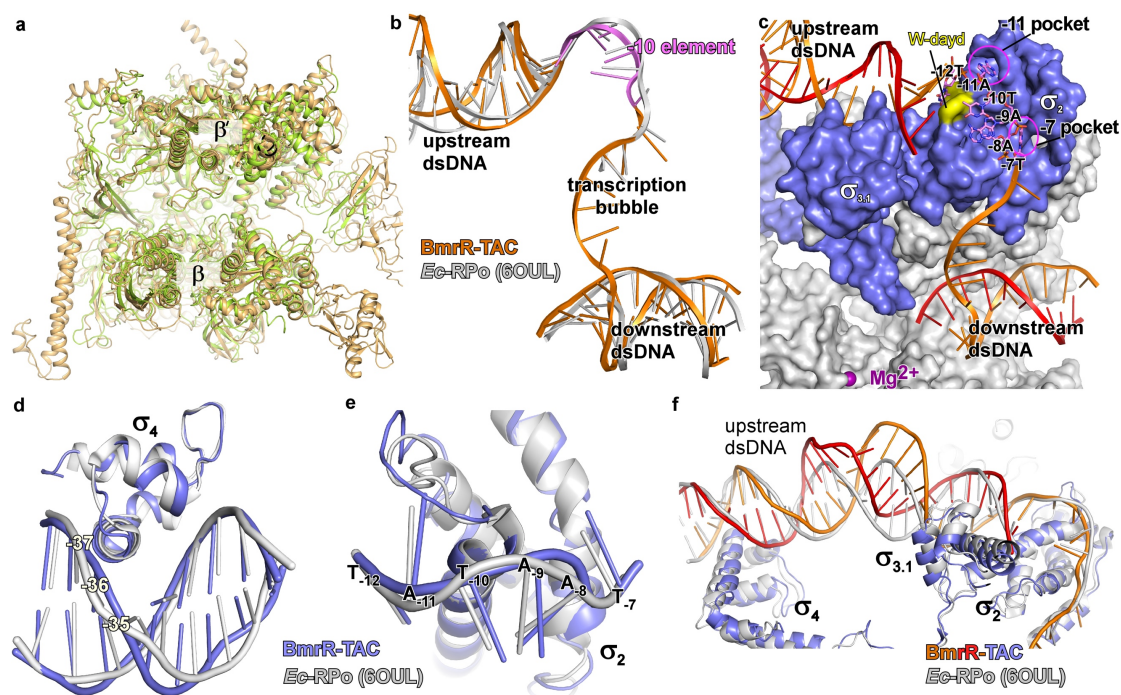
Supplementary Figure 1. The complex assembly of *B. subtilis* BmrR-TAC. (a) The promoters used in this study. *Pbmr*-1 was used for cryo-EM structure determination of BmrR-TAC and for fluorescence-detected *in vitro* transcription assay in (h); *Pbmr*-2 and *Pbmr*-wt1 (a wild-type *Pbmr*) were used for the gel-shift assay in (d); and *Pbmr*-wt2 (a promoter containing wild-type *Pbmr* sequence and a tR2 terminator) was used for radiochemical *in vitro* transcription assay in (c). The red box highlights regions with modified sequences. (b) The SDS-PAGE of endogenous *B. subtilis* RNAP holoenzyme. The experiment has been repeated >10 times. (c) The radiochemical *in vitro* transcription activity showing *B. subtilis* BmrR activates transcription from wild-type *Pbmr* (*Pbmr*-wt2) promoter in the presence of 0.5 mM tetraphenylphosphonium (TPP). The experiment has been repeated independently twice. (d) BmrR increases the formation of RPo from the wild-type (*Pbmr*-wt1) and derivative of *Pbmr* (*Pbmr*-2) promoters in the presence of 0.5 mM TPP. The experiment has been repeated independently three times. (e) The elution peak of *B. subtilis* BmrR-TAC from the Superose 6 size-exclusion column. (f) The SDS-PAGE of the *B. subtilis* BmrR-TAC, and BmrR. The experiment has been repeated twice. (g) The native-PAGE of the *B. subtilis* BmrR-TAC stained with Coomassie Brilliant Blue or SYBR Gold dye. The experiment has been repeated twice. (h) The fluorescence-detected *in vitro* transcription assay showing the reconstituted BmrR-TAC for cryo-EM study is able to extend 5' Cy3-labeled CGA trinucleotide in the presence of CTP. The identity of Cy3-CGA and Cy3-CGAC was further confirmed by LC-MS/MS. The slower migration of 3-nt RNA oligomer compared with 4-nt RNA oligomer has also been observed in primer-dependent transcription initiation using a 5'-OH RNA primer²³. The experiment has been repeated 3 times.



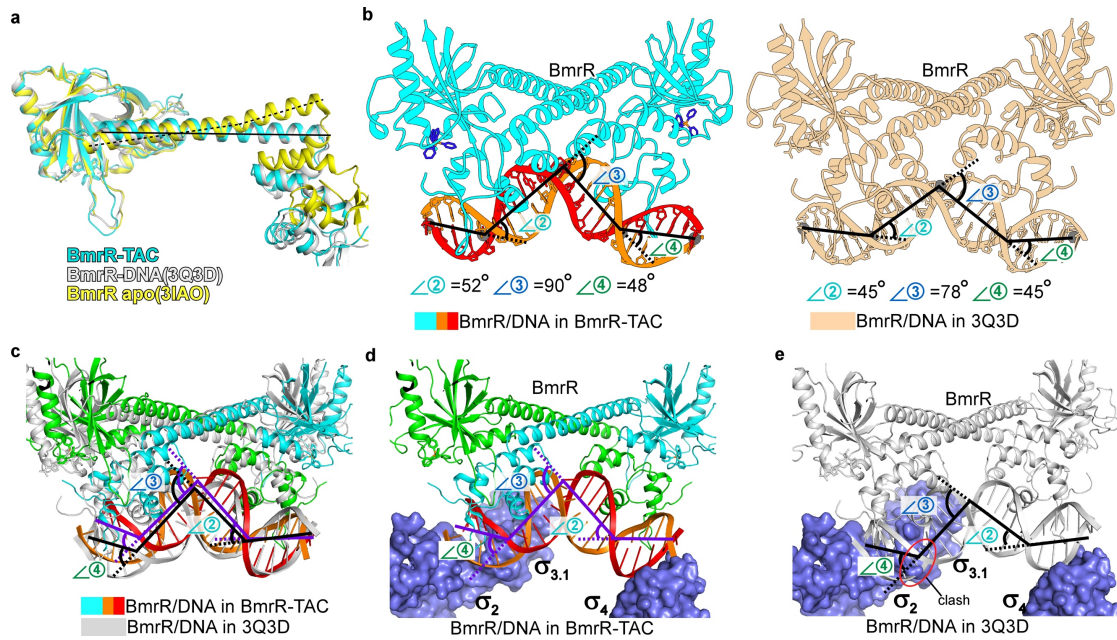
Supplementary Figure 2. The processing pipeline for single-particle reconstitution of cryo-EM map of *B. subtilis* BmrR-TAC. (a) The flowchart of data processing. **(b)** The angular distribution of *B. subtilis* BmrR-TAC single-particle projections colored by number of particles of each projection. **(c)** The gold-standard FSC of *B. subtilis* BmrR-TAC model reconstruction. The 0.143 FSC cutoff indicates a nominal resolution of 4.40 Å. **(d)** The cryo-EM map of *B. subtilis* BmrR-TAC colored by local resolution (calculated by Resmap).



Supplementary Figure 3. The interaction between RNAP-ε subunit and the rest of RNAP core enzyme. (a) The overall structure displays the location of RNAP-ε subunit on RNAP. **(b)** The RNAP-ε subunit makes extensive interaction with RNAP-α, β and β' subunits.



Supplementary Figure 4. The BmrR-TAC has characteristics of RPo. (a) The BmrR-TAC structure exhibits the same closed conformation of RNAP-clamp domain as that in *E. coli* RPo complex (PDB: 6CA0; ²⁴). (b) The BmrR-TAC structure exhibits the same DNA conformation of the transcription bubble and the downstream dsDNA (PDB: 6OUL; ²⁵). (c) The *B. subtilis* RNAP-holoenzyme accommodates the transcription bubble and downstream dsDNA in a similar manner as other bacterial RNAP. The W-dyad separates the upstream junction and the -11 and -7 pockets accommodate flipped bases in the structure. (d) The σ_4 /-35 interaction in the BmrR-TAC is similar to *E. coli* σ_4 /-35 DNA interaction (PDB: 6OUL; ²⁵). (e) The σ_2 /-10 interaction in the BmrR-TAC is similar to *E. coli* σ_2 /-10 DNA interaction (PDB: 6OUL; ²⁵). (f) The locations of σ_2 and σ_4 on RNAP core enzyme in BmrR-TAC are similar compared with the *E. coli* transcription open complex (PDB: 6OUL; ²⁵) with r.m.s.d. values of 1.95 Å for all σ_2 / σ_4 C α atoms of the two structures.



Supplementary Figure 5. The comparison of the BmrR-TAC structure with previous reported crystal structures. (a) The central helix adopts the straight conformation for activation in BmrR-TAC (cyan) as in the structure of BmrR-DNA (PDB: 1EXI; gray) compared with the inactive bent conformation in the BmrR apo structure (PDB: 3IAO). **(b)** Kinks 2, 3, and 4 pre-exist in the binary structure of BmrR-DNA (PDB: 3Q3D) and are further enlarged upon RNAP engagement. **(c)** The BmrR-DNA (PDB: 3Q3D) superimposed based on the -35 element with BmrR-TAC show slightly different paths of the downstream dsDNA due to different kink angles resulting in a steric clash of the promoter DNA with σ as shown in (e). **(d)** The σ_4 and $\sigma_{3.1}$ together induce slight enlargement of kinks 2, 3, and 4 in BmrR-TAC. **(e)** Structure modeling of BmrR-DNA (PDB: 3Q3D) onto RNAP holoenzyme, based on -35 element superimposition of BmrR-DNA and BmrR-TAC, implicates a steric clash between the promoter DNA and σ , suggesting the necessity of slight enlargement of the three kinks during promoter engagement of RNAP holoenzyme.

Supplementary References

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