Supplementary Information

Determination of RNA polymerase binding surfaces of transcription factors by NMR spectroscopy

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Supplementary Table 1: Coordinates of the β flap tip helix in the modeled NusA-NTD$^{\Delta}$:*Tt*RNAP complex. The table is an extract of the PDB file of elongating *Tt*RNAP (residues 767-781 of the β subunit; PDB ID: 2O5I) docked to NusA-NTD$^{\Delta}$ as described in the Material and Methods section, giving the position of *Tt*RNAP relative to the deposited coordinates of NusA-NTD$^{\Delta}$ (PDB ID: 2KWP).

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ATOM  10777  N   PRO C 767       3.453  -1.849 -42.571  1.00 90.52           N
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ATOM  10783  CD  PRO C 767       4.098  -3.134 -42.897  1.00 90.44           C
ATOM  10784  N   THR C 768       2.013  -1.418 -39.212  1.00 92.37           N
ATOM  10785  CA  THR C 768       2.261  -0.517 -38.082  1.00 93.42           C
ATOM  10786  C   THR C 768       2.739  -1.281 -36.849  1.00 94.34           C
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ATOM  10829  C   LEU C 773      -1.069  -5.199 -30.435  1.00 96.83           C
ATOM  10830  O   LEU C 773      -1.557  -6.174 -29.874  1.00 97.78           O
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ATOM  10889  CD  GLU C 780      -2.821 -14.257 -35.186  1.00 97.77           C
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ATOM  10897  CG  LYS C 781      -7.331 -14.173 -36.226  1.00 93.86           C
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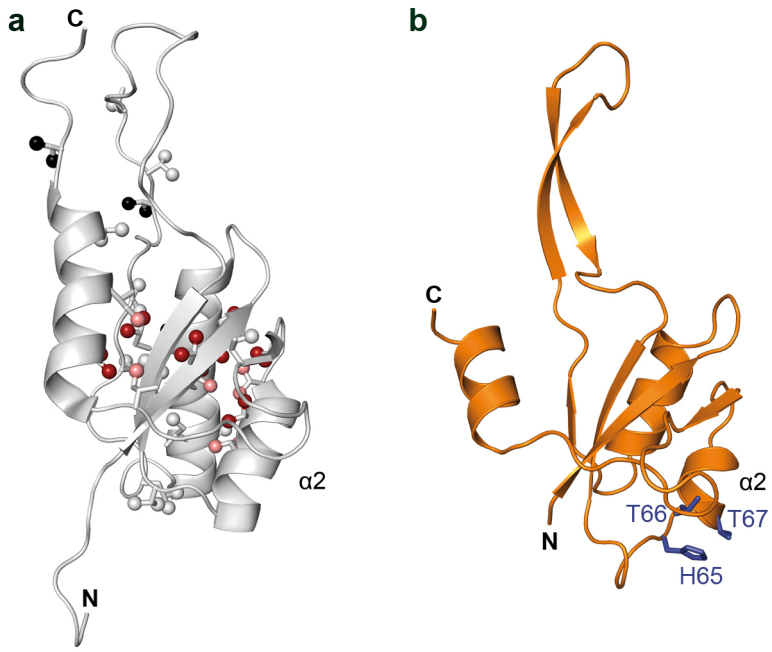
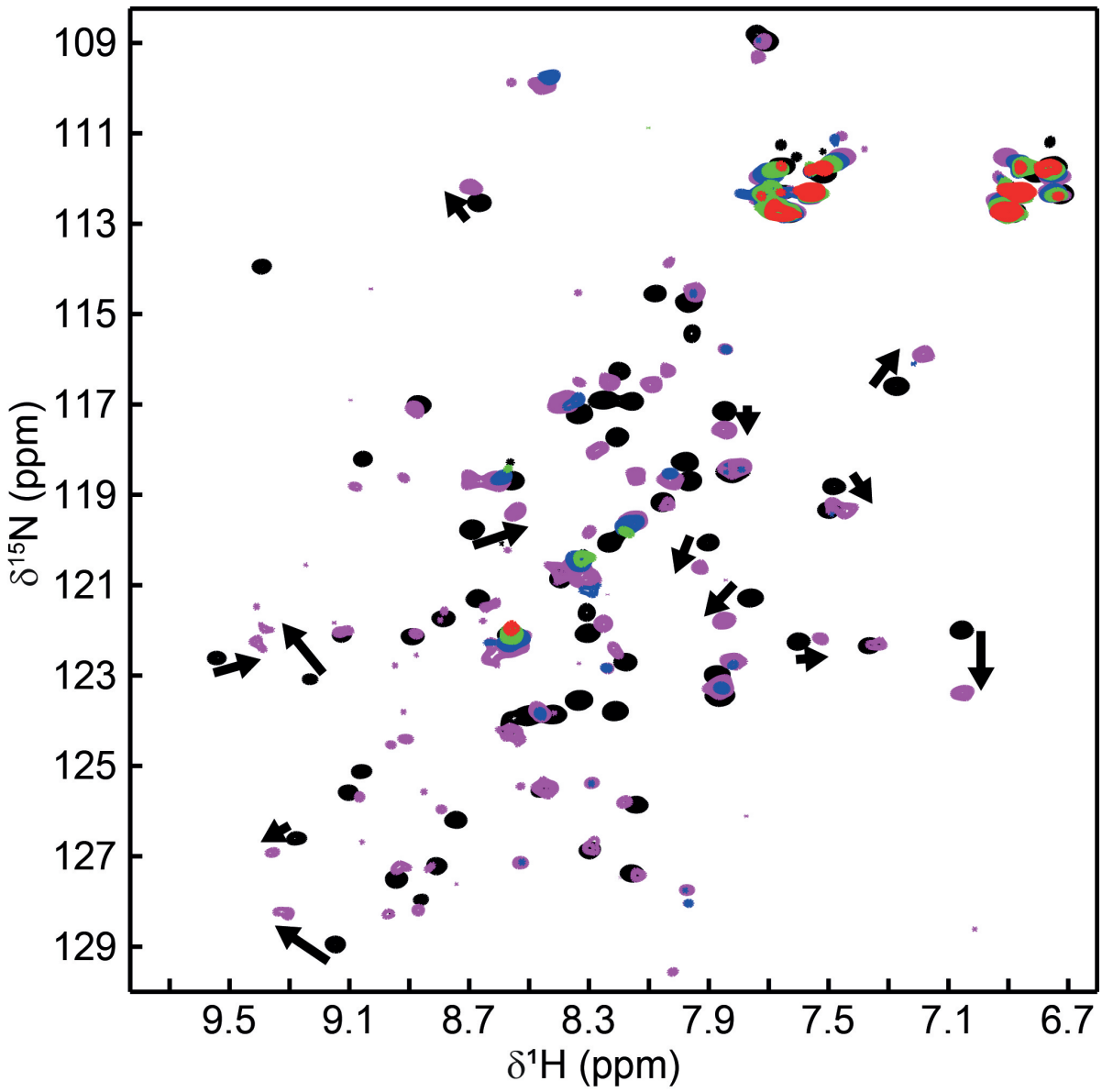
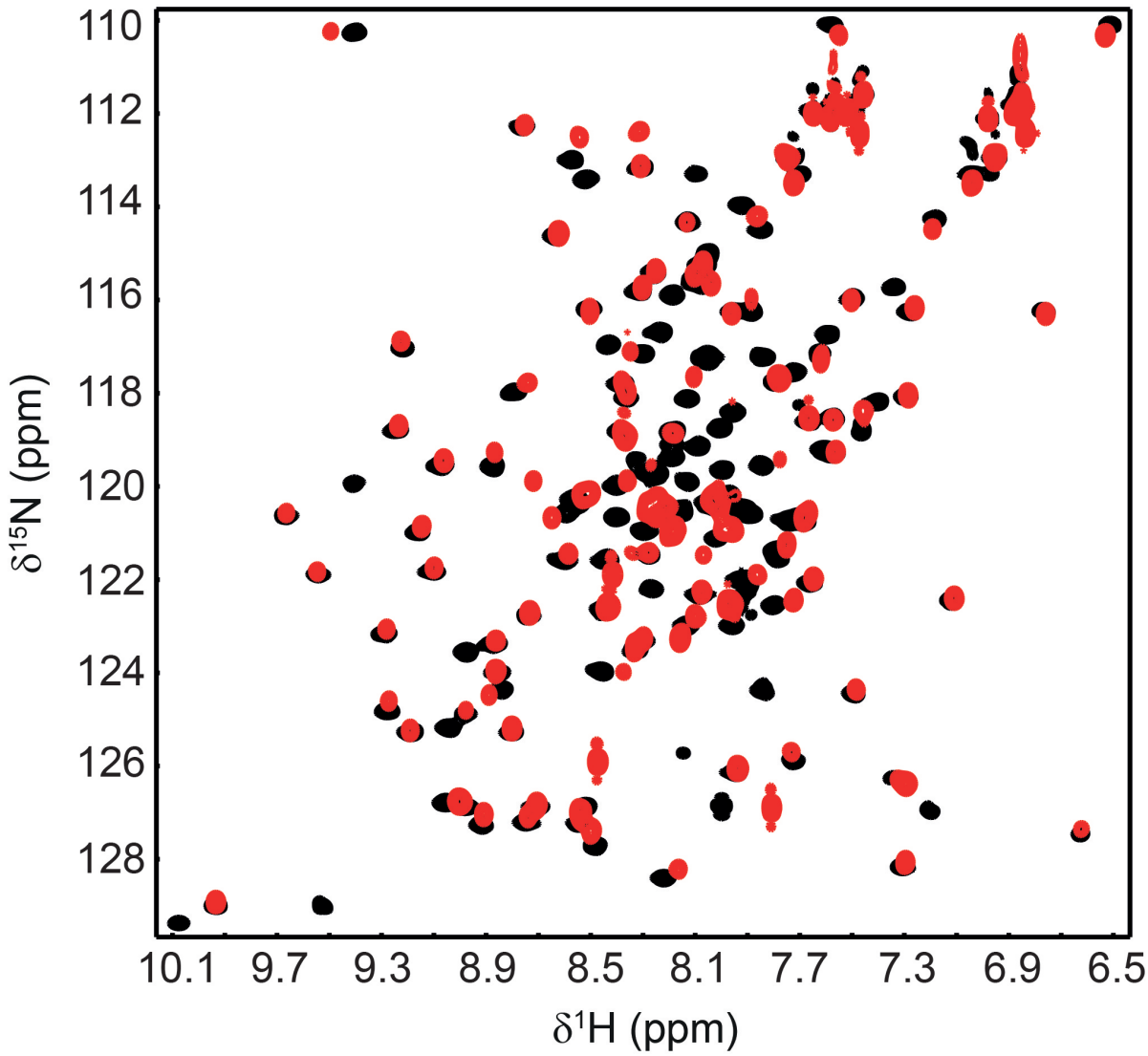


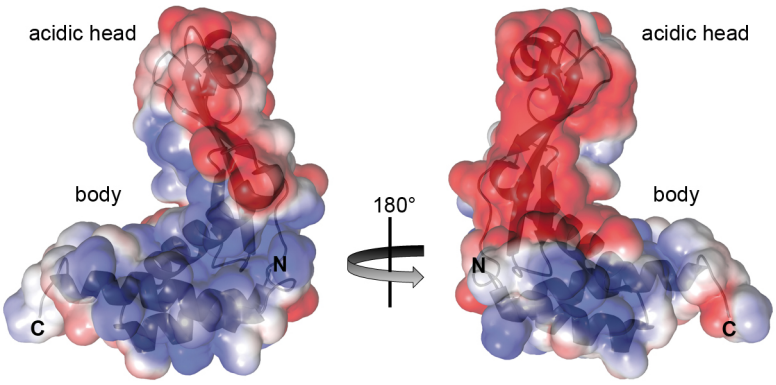
Supplementary Figure 1: Structures of NusG-NTD and RfaH-NTD. (**a**) RNAP binding site of NusG-NTD. Structure of NusG-NTD (PDB ID: 2K06) in cartoon representation, grey. Ile, Leu, and Val residues are shown as sticks with the carbon atoms of their methyl groups represented as spheres. Strongly affected methyl groups, dark red; slightly affected methyl groups, light red; unaffected methyl groups, grey; unassigned methyl groups, black. (**b**) βGL binding motif of RfaH-NTD. Structure of RfaH-NTD (PDB ID: 2OUG) in cartoon representation, orange. Residues involved in βGL binding are shown as blue sticks and labeled.



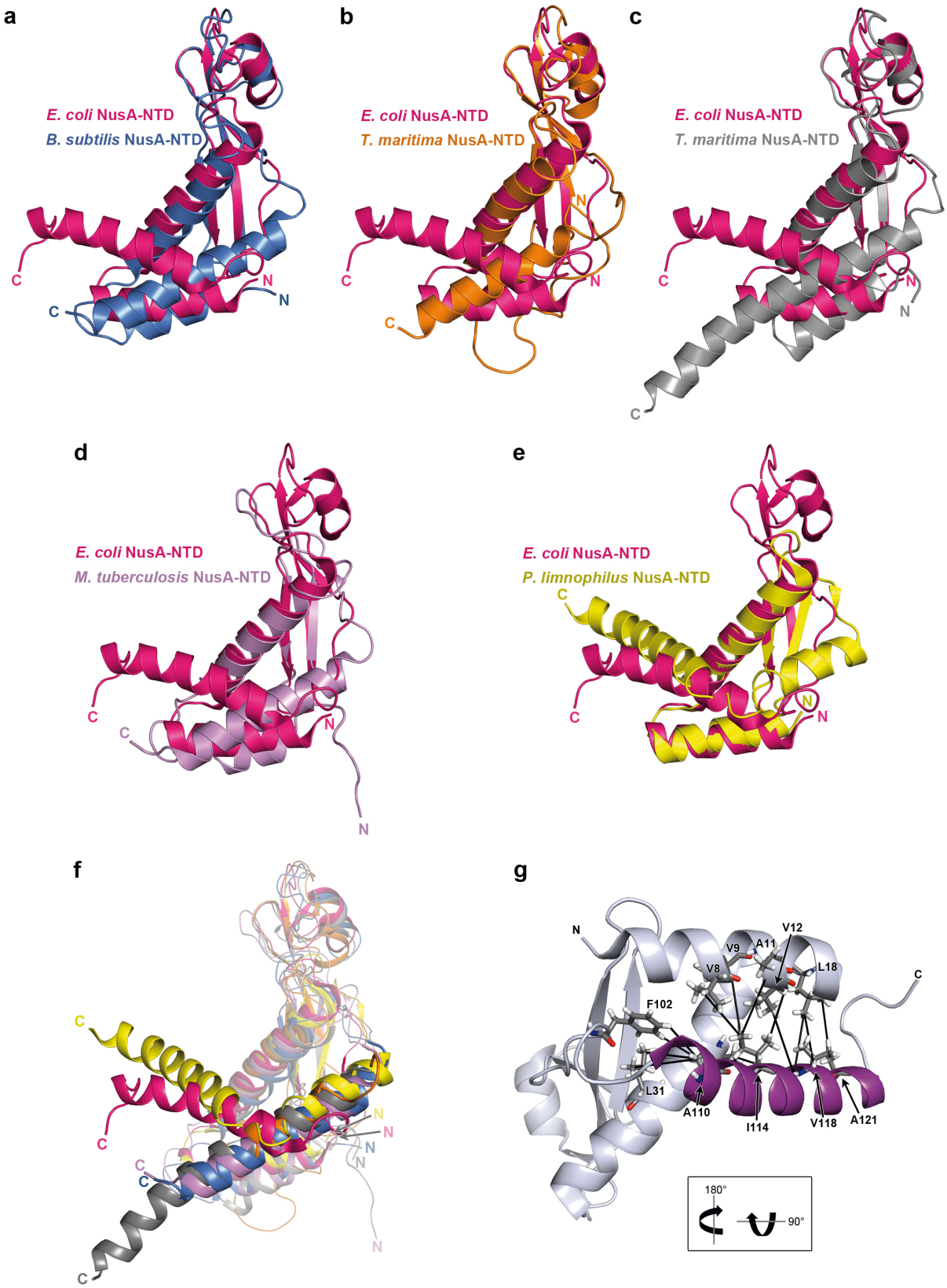
Supplementary Figure 2: Displacement of RNAP from NusE$^{\Delta}$ by NusG-CTD. 2D [^{1}H,^{15}N]-HSQC spectra of free NusB:[^{15}N]-NusE$^{\Delta}$, black, NusB:[^{15}N]-NusE$^{\Delta}$ in the presence of RNAP in equimolar concentration, red, and NusB:[^{15}N]-NusE$^{\Delta}$ in the presence of RNAP and NusG-CTD (molar ratio 1:1:1, green; 1:1:3, blue; 1:1:10, purple). Black arrows indicate the chemical shift changes that occur upon complex formation of NusG-CTD and NusB:[^{15}N]-NusE$^{\Delta 1}$.



Supplementary Figure 3: Superposition of the [^{1}H,^{15}N]-HSQC spectra of [^{15}N]-NusA-NTD(1-137), red, and [^{15}N]-NusA-NTD$^{\Delta}$, black. The protein concentration was 400 µM in each sample.



Supplementary Figure 4: Electrostatic potential molecular surface of NusA-NTD$^{\Delta}$. NusA-NTD$^{\Delta}$ in cartoon and surface representation. The electrostatic surface potential is colored from -2 kT/e, red, to +2 kT/e, blue.



Supplementary Figure 5: Comparison of NusA-NTD structures. (**a-e**) Superposition of NusA-NTD$^{\Delta}$ (pink) with (**a**) *Bs*NusA-NTD (blue, PDB ID: 2MT4, root mean square deviation

(r.m.s.d.) 1.8 Å), (**b**) *Tm*NusA-NTD (orange, PDB ID: 1HH2, r.m.s.d. 1.9 Å) (**c**) *Tm*NusA-NTD (grey, PDB ID: 1L2F, r.m.s.d. 1.7 Å), (**d**) *Mt*NusA-NTD (violet, PDB ID: 1K0R, r.m.s.d. 1.8 Å), and (**e**) *Pl*NusA-NTD (yellow, PDB ID: 4MTN, r.m.s.d. 1.4 Å). The linker helix was not used for the superpositions. **(f)** Superposition of NusA-NTD structures shown in (**a-e**). The linker helix is shown in bright colors. **(g)** NOE network fixing the position of the linker helix in NusA-NTD$^{\Delta}$ (cartoon representation, grey; the linker helix is highlighted in purple). The inset indicates how the molecule is rotated in respect to (**a**). Residues participating in the NOE network are labeled and shown as sticks (carbon atoms, dark grey; nitrogen atoms, blue; oxygen atoms, red; hydrogen atoms, white). Unambiguously identified NOEs are shown as black lines. For clarity only one NOE is displayed per methyl group (using the corresponding methyl carbon atom as center).

Supplementary References

1. Burmann, B. M. *et al*. A NusE:NusG Complex Links Transcription and Translation. *Science* **328**, 501-504 (2010).