ESRF	**Experiment title:** *Mycobacterium smegmatis* RNA polymerase transcription machinery (MycPol)	**Experiment number**: MX 2182
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Report:

***Mycobacterium smegmatis* RNA polymerase transcription machinery**

In bacteria, a single RNA polymerase (RNAP) is responsible for all transcription. Transcription initiation is a major control point of gene expression. Structures of RNAPs and their complexes with diverse transcription regulators are crucial for understanding RNAP function at the molecular level. RNAP from *Mycobacterium smegmatis*[1] (*Msm*) is a valid transcription model system of its close pathogenic relatives, such as *Mycobacterium* tuberculosis. Importantly, RNAP from these human pathogens is a proven drug target [2].

We have obtained three cryo-EM structures (two at ~3.1 Å) of *Msm* RNAP in complex with its interaction partner, a helicase-like factor termed HelD[3]. We revealed that HelD has crescent-like shaped and simultaneously penetrates deep into two RNAP channels, one responsible for DNA binding, and the other for NTP entry to the active site. This HelD-RNAP interaction is incompatible with transcription. Furthermore, HelD prevents non-specific interactions between the RNAP core and DNA and is able to displace RNAP from stalled

elongation complexes but does not prevent σ factor binding to RNAP. Our results thus define HelD as a clearing factor that rids RNAP of nucleic acids and provide insights into the architecture of the highly medically relevant mycobacterial transcription machinery. The work has been published as a preprint at the biorxiv (https://doi.org/10.1101/2020.07.20.211821) together with two accompanying papers and it is currently under revision in Nature Communications.

The cryo-EM data was collected on ESRF CM01 and CIETEC on the proposed HelD-RNAP complex sample. Three main 3D classes of the target RNAP-HelD complex (Figure 1) were identified and refined to 3.1 Å, 3.1 Å and 3.5 resolution, respectively, at the 0.143 gold standard Fourier Shell Correlation (FSC) cut off (Figure 2).

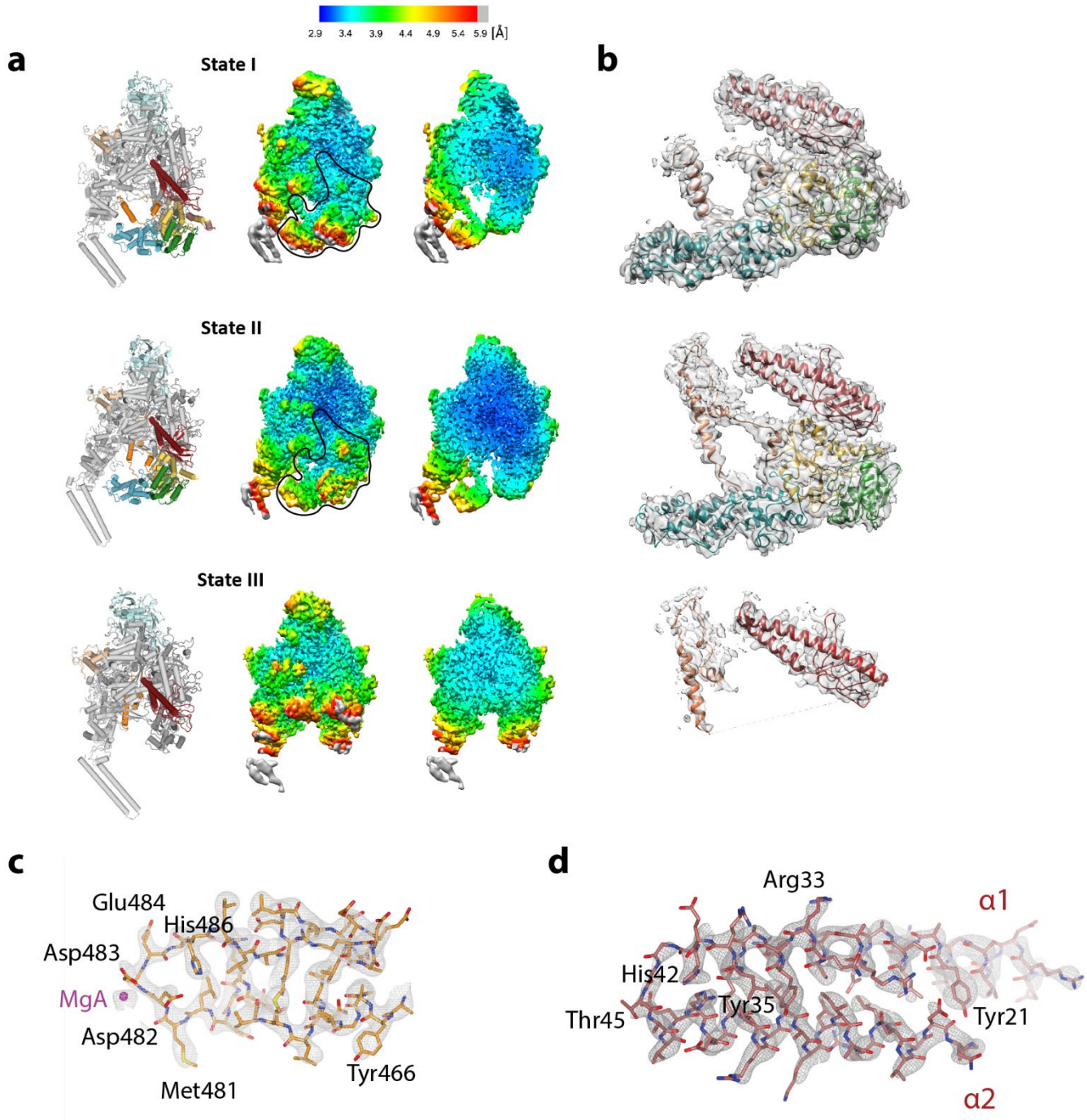

Figure 1: Resolution analysis of the *Msm* HelD-RNAP complex

a, three identified states of *Msm* HelD-RNAP complex, (**left**) atomic models, (**right**) local resolution. **b**, fit of the atomic models of HelD protein into cryo-EM maps. **c** and **d** fit of selected areas of HelD protein into cryo-EM maps

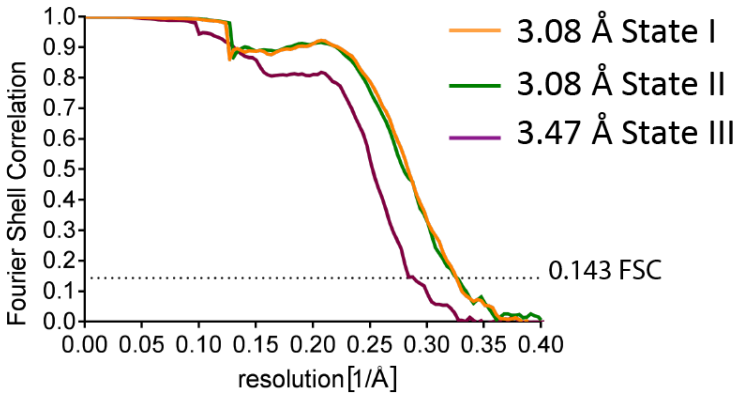

Figure 2: Estimated resolution of the three identified states of *Msm* HelD-RNAP complex at the 0.143 gold standard Fourier Shell Correlation (FSC).

References

1 Kouba, T. *et al.* The Core and Holoenzyme Forms of RNA Polymerase from Mycobacterium smegmatis. *J Bacteriol* **201**, doi:10.1128/JB.00583-18 (2019).

2 Ma, C., Yang, X. & Lewis, P. J. Bacterial Transcription as a Target for Antibacterial Drug Development. *Microbiology and molecular biology reviews : MMBR* **80**, 139-160, doi:10.1128/MMBR.00055-15 (2016).

3 Wiedermannova, J. *et al.* Characterization of HelD, an interacting partner of RNA polymerase from Bacillus subtilis. *Nucleic Acids Res* **42**, 5151-5163, doi:10.1093/nar/gku113 (2014).