Cryo-EM structures of the *M. smegmatis* RNA polymerase apoenzyme and the Sigma-A factor bound holoenzyme

T. Kouba[1], J. Pospíšil[2], H. Sanderová[2], A.R. Fersht[1], L. Krásný[2]
[1]Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom,
[2]Laboratory of Molecular Genetics of Bacteria, Institute of Microbiology, Academy of Sciences of the Czech, Prague, Czech Republic, **tkouba@mrc-lmb.cam.ac.uk**

In bacteria, the RNA polymerase (RNAP) is solely responsible for all transcription. Structures of RNAPs and their complexes with diverse transcription factors are crucial for understanding RNAP function and transcription initiation at the molecular level. The vast majority of mechanistic studies on bacterial transcription have used *Escherichia coli* (Eco) RNAP as a model. However, in many cases the properties of Eco RNAP are not necessarily representative of RNAPs from other bacterial species. Here we present 4.5 Å resolution cryo-electron microscopy structures of RNAP from *Mycobacterium smegmatis*, which is a valid biological model of its close pathogenic strain *Mycobacterium tuberculosis*. We show both the apoenzyme and the Sigma-A factor bound holoenzyme in multiple conformational states. We can distinguish three different orientations of the apoenzyme RNAP clamp switching between closed and open conformations. The structures of the holoenzyme show multiple positions of the σA2 region of σA factor towards the beta prime subunit clamp. Our structures of RNAP in *M. smegmatis* provide insights into the very early stages of transcription initiation and describe the enzyme movements during the process. The results establish an important cornerstone towards determining cryo-EM structures of further consecutive states of transcription pathway in this model organism.