The cryo-EM density map contains the full σ^A along with region 1.1. Being negatively charged (Schwartz et al., 2008), region 1.1 prevents access to the DNA to enter the active site channel (Murakami, 2013). There are differences in the orientation as well as positioning of the region 1.1 in *E. coli* as well as *Mtb* RNAP. Region 1.1 in *E. coli* is located within the active site channel whereas it is displaced outside the active site channel in the *Mtb* RNAP open promoter complex. In *E. coli*, σ region 1.1 is oriented perpendicular to the DNA binding site whereas in *Mtb*, it is oriented parallel to the floor of DNA binding site. Furthermore, there is a taxon-specific sequence insertion in the *Mtb* β' subunit which is α-helical and adopts a coiled coil conformation (Lin et al., 2017) (Figure 5.9C). The detailed structural analysis will be made once the refinement of the structure is finished.

5.4 Summary and future implications

During the Newton Bhabha PhD placement programme, we were successful in optimizing the protein purification and the samples suitable for TEM studies using negative staining and cryo-EM techniques. We were also successful in solving the single particle cryo-EM structure of RNAP bound to principal sigma factor, σ^A. Although, there are several high resolution crystal structures of bacterial RNAPs available in PDB, this is the first solution structure of bacterial RNAP holoenzyme solved at such a high resolution (4.2 Å). The preliminary structure analysis of RNAP reveals that it is a comprising of three *E. coli* subunits i.e. ββ'ω and two subunits from *Mtb* i.e. σ^A and α_2, hence the solved structure is of a chimeric RNAP holoenzyme.

The presence of the full length σ^A bound to the RNAP will help us in identifying conserved RNAP/σ-factor protein-protein interaction hotspots that can be targeted for drug design. The positioning of $\sigma^A_{1.1}$ in the holoenzyme is crucial for the transcription initiation, hence RNAP holoenzyme structure will allow us to visualize structural conformation of the region 1.1 before binding promoter elements. Structural comparison with the initiation complex will help us map the conformational changes upon binding promoter elements in the conserved regions of σ^A and regions of RNAP involved in binding σ^A. One more important aspect of the project was to solve structure of RNAP bound to

accessory protein RbpA which confers rifampicin and stress tolerance to bacteria (Newell et al., 2006a). We were also successful in optimizing the conditions suitable for performing TEM studies using negative staining and cryo-EM techniques for holoRNAP (σ^A) bound to RbpA. We could collect and process data for RNAP bound to RbpA and solve structure to 12 Å resolution. The presence of high salt concentration in the samples, which is necessary to keep RNAP stable, contributed to the high background and low signal/noise ratio. Due to time constraints, we could not perform more experiments to achieve high resolution solution structure for the complex. The key findings of this study will provide crucial insights to aid structure guided drug design and may help us better understand the conformational rearrangements happening in the RNAP holoenzyme upon binding promoter elements at the initiation step.