Summary

TetR family regulators play vital roles in survival and pathogenesis of bacterial pathogens. *Mtb* genome encodes for ~50 TetR family regulators but only few have been characterized so far. *Mtb* also displays a complex lipid repertoire and possesses a battery of genes to regulate complex lipid metabolism. Many TetR family regulators, sub-grouped as FaDRs, control the expression of genes involved in lipid metabolism but no FaDR equivalent has been characterized from *Mtb* so far. In this study, we report for the first time, on the detailed structural and biophysical characterization of putative TetR family, belonging to FaDR subgroup, *Rv2506* gene product, which we named as FaD35R, from *M. tuberculosis* (*H37Rv*). Purified FaD35R is a homodimer in solution, binds tetracycline family antibiotics, activated fatty acids, and citrate. Results show that ligand binding alters its DNA binding properties and also promotes high oligomerization, as evidenced by isothermal titration calorimetry, fluorescence and particle size analysis studies. Results of equilibrium and kinetic studies show that FaD35R binds to the FaD35R-box with very high affinity and specificity. The binding of FaD35R to DNA was abolished in the presence of activated long chain fatty acids like PCA. Further, we resolved the first crystal structure of Fad35R at 3.4 Å. Each monomer comprises of N-terminal DNA binding domain (DBD) and C-terminal ligand binding domain (LBD). Interestingly, arrangement of homodimer does not follow canonical-symmetric dimer where both monomers are inverted-mirror images of each other. Structural comparison with DNA-bound state of *Pseudomonas aeruginosa* DesT shows that DBD of one monomer is in bound-state whereas the other DBD is in unbound-state, suggesting that FaD35R structure may mimic the structure of intermediate during promoter binding. The ligand binding pocket is also in open state and each binding site is accessible from opposite side of the dimer. This reverse polarity in ligand access suggests that different ligands may trigger conformational changes with different magnitudes and remodel

DNA binding site differently. Analysis of symmetry-mates indicated that alternative quaternary states may be accessible to FaD35R. To test this, we examined the ligand induced oligomerization and results indicate that the assembly state of FaD35R is sensitive to ligand concentrations. Ligand induced changes in assembly-state and alternative quaternary states observed in this study indicate that promoter recognition properties of FaD35R would be controlled through multiple mechanisms which may be ligand specific. Together, our results present a first detailed structural and biochemical characterization of FaD35R from *M.tuberculosis*. Our results reveal non-canonical structural features of FaD35R that are important in ligand and DNA binding, and in addtion, reveal alternative quaternary states that are accessible upon ligand binding.