Signalling is an important event for growth and survival of any microbe, especially for successful pathogens, where they have to overcome host defense system. In this consequence, utilization of protein phosphorylation as a molecular switch in the process of growth of a successful pathogen, *M. tuberculosis* has been envisaged. The involvement of protein kinase(s) and phosphatases in the phosphorylation/dephosphorylaton of protein is well known. In fact, analysis of *M. tuberculosis* genome sequence revealed the presence of eleven eukaryotic-type Ser/Thr kinases and a cognate phosphatase. Among them, the role of kinases like PknA/ PknB and the only phosphatase, PstP in cell growth have been assigned; however, the mechanism of kinase activation/deactivation, which drives its functionality, has not yet been elucidated. In this scenario, the major focus of the work embodied in this thesis is on PknA, an essential mycobacterial eukaryotic-type Ser/Thr kinase, which has already been characterized (Chaba et al, 2002; Thakur et al, 2008; Ravala et al, 2015). The involvement of three threonines in the activation loop of PknA at amino acid residues 172, 174 and 180 in auto- and trans-phosphorylation activity of the protein has already been established (Ravala et al, 2015). Nevertheless, the activation mechanism of this Ser/Thr kinase is not known and the embodied work deals with this aspect by employing *in silico* approach.

The characterization of the catalytic domain of a kinase is important in understanding the mechanism of activation. The N - terminal 283 amino acids of PknA (PknA-283) were found to exhibit auto-phosphorylation activity comparable to that of the full length protein (431 amino acids; Fig 3.2). Further, in analytical ultracentrifugation, PknA-283 was found to be a monomer (Fig 3.4). The role of Thr172, Thr174 and Thr180 was highlighted in transphosphorylation of a substrate mGmk (guanylate monophosphate kinase) by PknA (Fig 3.6). In contrary, T172AT174A mutant showed negligible transphosphorylation of mGmk (Fig.3.6). T180A mutant also did not show transphosphorylation of mGmk (Fig 3.6.). Subsequently the model of PknA-283 was generated since amino acid residues 84-88 were missing in the crystal structure (PDBID: 4OW8). Molecular dynamics simulations with PknA model revealed that the average RMSD of unphosphorylated form of PknA (2.7Å) was higher than that of the phosphorylated counterpart. For PknA phosphorylated at Thr172, Thr174 and Thr180, the average RMSD was 2.1Å, 1.9Å and 2.6Å respectively. Root mean square fluctuation (RMSF) values of activation loop residues Pro179 to Ala189 were lesser for PknA phosphorylated at Thr180 as compared to unphosphorylated PknA (Fig 3.10.). Thr180 showed proximity to the catalytic residue Asp141 as compared to Thr172 (15-22Å) and Thr174 (13-20Å) in unphosphorylated condition (Fig 3.11). On the other hand, in phosphorylated condition, Thr172 and Thr174 moved further away from Asp141 to distances up to 24Å (Fig.3.11). In unphosphorylated PknA, the distances between Oγ atoms of Thr172, Thr174, Thr180 and γ phosphate of ATP were 17-22 Å, 13-20Å and 8-15Å, respectively (Fig 3.12.). In phosphorylated condition, Thr180 came closer to ATP (9.5Å). Further, a salt bridge was formed between Lys143 and Asp141 in both phosphorylated and unphosphorylated PknAs. In addition, there was interaction between side chain of Thr180 and Lys143 particularly in phosphorylated PknA, involving movement of activation loop (Fig 3.13). Thus, these observations were indicative of cis phosphorylation of PknA as Thr180 came closer to ATP, followed by trans-phosphorylation of Thr172 and Thr174.

The activation of a kinase is accompanied by its subsequent deactivation through dephosphorylation. In this context, the unique Ser/Thr phosphatase of *M. tuberculosis*, PstP plays an important role. Its interaction with different Ser/Thr kinases has been analysed. Docking studies revealed that a few of the N terminal residues such as Val7 to Tyr10, Ala11 to Ser14, Val27 to Ala31, Gln51, Ala55, Ala58, His59, Asp61 and Asp62 of PstP are in the interface of two or three types of the docked complexes with kinases i.e. PknA, PknB and PknG (Table 4.1). Lesser number of residues (Pro157 to Arg159, Leu161, Met163, Glu210, Ala212, His216 and Glu239) have been found to be common in the C-terminal of PstP in docked complexes involving maximum of three kinases. The complexes of PstP with PknE showed relatively distinct interface; however showing a few common residues at interface with those with PknA and PknB (Table 4.1).

Autophosphorylation of a kinase usually leads to transphosphorylation of its substrate(s), which is a crucial to its functionality. Docking studies with PknA and its substrates like FtsZ and phosphodiesterase indicated the importance of N terminal residues from Met1 to Val5, Arg17 to Thr21, Gln26, Trp28 and Lys45 in the interactions with substrates. In addition, other common residues of PknA in interactions with these two substrates (FtsZ and phosphodiesterase) are Asn104, Ser105, Lys108,Met176 to Gly179 and Gly221 to Ala222 (Table 4.2). Amino acid residues- Met67 and Ala135 of PknA have been found as common residues at the interface of docked complexes with GarA and Gmk (Table 4.2). Few residues from the C - terminal of PstP have been found at the interface of docked complexes with its substrates under this study. Even though no conserved stretch of amino acids has been obtained across all docked complexes, the aforesaid residues of PknA or PstP could be experimentally validated as important residues for interaction. The selection of a small proportion of the 2000 docked complexes obtained for every pair of proteins matched, could be a factor in not getting a conserved motif. Such a motif could also be obtained if the cutoff of the occurrences of amino acids at the interface of selected complexes is decreased.

Finally, the work carried out in this study unravels the mechanism of activation of PknA, an essential Ser/Thr kinase of *M. tuberculosis* that is involved in growth of the bacterium. This work also highlighted the possibility of interaction of PknA with the unique Ser/Thr phosphatase PstP. Further, the interaction of PknA with its substrates has also been studied. The substrates include FtsZ, a protein of pivotal importance for cell division. It is involved in cell growth and is known to form the septum at midcell, with other accessory proteins. Thus, residues involved in deactivation of PknA and those that are involved in transphosphorylation of substrates have been identified *in silico* and may be experimentally validated. The importance of the work is highlighted by the fact that PknA is a putative drug target and study of its activation, deactivation and interaction with substrates could provide a platform for screening of small molecule libraries by novel means in quest of new anti-mycobacterial compounds.