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STRUCTURAL ANALYSIS OF PKNA PROTEIN IN *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Mycobacterium tuberculosis contains 11 serine threonine kinases which includes PknA and PknB. PknA is a secreted protein of *M. tuberculosis.* PknA is identified as one of the most interacting protein in our protein-protein interaction study between *M. tuberculosis* and human. Primary structure prediction and physicochemical analysis of PknA was carried out by ProtParam. Comparative study of the secondary structure of PknA using GORIV and SOPMA revealed a high percentage of alpha helix and coiled coils than beta sheets. In the present study, we predicted PknA tertiary structure by homology modelling because its structure is not yet revealed by any experimental methods. The structural analysis will help in the functional study of the protein.

Key words: PknA, Homology Modeling, Serine/ Threonine Kinase, *M. tuberculosis*, Procheck Validation

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is one of the world's most devastating human pathogens. In 2004, 49 million people developed active TB and approximately 2 million people died from it, making this disease the second leading cause of infectious disease mortality worldwide **[1]**. TB is the leading cause of

death in HIV-infected individuals. Infection with HIV increases the risk of TB and also increases the risk of reactivating latent disease to over 20 times that in HIV-negative people as immunosuppression worsens **[2, 3]**.

M. tuberculosis has an array of proteins to ensure its existence during the course of infection. In order to thrive and maintain its homeostasis, the pathogen continuously influences its surroundings mainly through surface-located sensor proteins. Extracellular signals are communicated through the sensors to the cytosol leading to the appropriate cell responses. Apparently, a large number of pathogens employ reversible phosphorylation of proteins by kinases and phosphatases as a way of transmitting the signals from extracellular milieu which helps in their survival and pathogenicity **[4-7]**. Kinases carry out the phosphorylation by transferring the phosphate moiety on target proteins and phosphatases convert them back to the unphosphorylated state, either by dephosphorylating the substrate or by regulating the activity of kinases.

The coordinated regulation of Ser/Thr protein kinases (STPKs) and phosphatases is essential for maintaining the appropriate equilibrium of protein phosphorylation. Membrane associated kinases and phosphatases are known or hypothesized to be regulated by external stimulus. Kinases are attractive as drug targets due to the range of crucial cellular processes in which they are involved. The *M. tuberculosis* (STPKs) are attractive targets partly because of the inferred importance of serine/threonine phosphorylation in *M. tuberculosis: M. tuberculosis* is unique within the bacterial world in having a much higher number of STPKs compared to the more common two-component signalling systems **[8-11]**.

Protein phosphorylation mediated by receptor Ser/Thr protein kinases (STPKs) is widely used to transduce extracellular signals into intracellular responses. Ser/Thr phosphorylation prompts numerous, broad effects--including changes in transcription, metabolic flux, cell growth, cell division, protein localization, and immune defense/pathogenesis **[12, 13]** each requiring precise regulation of kinases for accurate signal transduction. PknB, a member of the newly described eukaryotic-like serine/threonine kinase family from *M. tuberculosis* **[14, 8]**. *M. tuberculosis* encodes in its genome 11 putative serine/threonine kinases In contrast, *E. coli* and other bacteria whose genomes have been completely sequenced thus far do not contain this family of proteins. In *M. tuberculosis*, STPKs have

been estimated to phosphorylate several hundred proteins **[15]**.

While two of the 11 *Mtb* STPKs are soluble kinases, nine are predicted transmembrane receptors with an N-terminal "eukaryotic-like" kinase domain (KD) linked through a single transmembrane helix to an extracellular sensor domain **[9]**. Orthologs of the *Mtb* transmembrane receptor Protein Kinase B (PknB) are the most widely distributed STPKs in the prokaryotic kingdom. PknB is essential for *Mtb* growth **[16, 17]** and it phosphorylates diverse substrates, including proteins involved in peptidoglycan synthesis **[18]**, cell division **[19]**, the stress response **[12]**, transcription **[13]**, metabolic control **[20]**, and other STPKs **[21]**. A key challenge is to understand how bacterial receptor STPKs such as PknB respond to extracellular signals.

The role of STPKs in post-translational modification and their further impact on regulating morphological changes associated with cell division have become important to use them as targets of pathogenesis. Changes in morphology and the observation that the kinases are highly expressed during exponential growth than in stationary phase suggest a role for the kinases in regulating active growth and shape determination **[22]**. The first evidence that PknA may regulate cell division came from the data showing that over-expression of *M. tuberculosis* PknA in *E. coli* results in elongated cells **[23]** The *PknA*-overexpressing strains form long, broad, and in some cases branched bacilli, with what appear to be incomplete septations, suggesting defects in cell wall synthesis and/or cell division. The *pknB*-over expressing strain forms widened, bulging cells of non uniform diameter, again suggesting effects on cell wall synthesis or cell division.

Primary structure prediction and physicochemical characterization were performed by computing theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY). The amino acid sequence provides most of the information required for determining and characterizing the molecule's function and physical and chemical properties.

Homology modeling combines and requires the techniques from two sets of biocomputing toolkits — sequence analysis and molecular modeling. We need at least a pair of sequences to do homology modeling. Multiple sequence alignments make the technique even more powerful. One member

of the dataset will be modeled, the others are reference sequences that have three-dimensional coordinate data associated with them upon which to base the model. Proteins of the same function generally have similar structure and this is the base for homology modeling. Homology modeling combines sequence analysis and molecular modeling to predict three dimensional structure. We have to choose a homologue of our query protein that has not had its structure yet solved and use the Swiss Model WWW resource to model the molecule. The theoretical structure is then visualized with Swiss-PDBViewer to gain insight into the way in which its structure relates to its function. An automated homology modeling tool is available on Bairoch's ExPASy server in Switzerland supported by Glaxo Smith Kline it is called SwissModel (http://www.expasy.org/swissmod/SWISS-MODEL.html **[24]**. This has dramatically changed the homology modeling process. It is a relatively painless way to get a theoretical model of a protein structure. It won't always generate a homology model for your sequence, depending on how similar the closest sequence with an experimentally solved structure is to it; however, it is a very reasonable first approach and will often lead to remarkably accurate representation.

MATERIALS AND METHODS

Primary Sequence Analysis of PknA

The sequence of *Mtb* PknA of accession P65726 was retrived from UniProt (http://www.uniprot.org/), a protein database, in FASTA format.

The primary structure, the basic physico-chemical properties, of PknA was analysed using ProtParam (http://web.expasy.org/protparam/ (Gasteiger E). ProtParam is available through the ExPASy server.

Secondary Structure Prediction

GORIV (http://npsapbil.ibcp.fr/cgibin/npsam) and SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was used to predict the secondary structure of PknA. GOR method is based on information theory **[25]** self-optimized prediction method (SOPM) **[26]** has been developed to improve the success rate in the prediction of the secondary structure of proteins.

Tertiary Structure Prediction

The modeling of the three dimensional structure of the protein was performed by Swissmodel homology modeling programs **[27]**. The SWISSMODEL depended on the quality of the sequence alignment by BLAST and template structure. Structural analysis was performed and figures representations

were generated with Swiss PDB Viewer **[24]**. Tertiary structure was predicted using homology modeling by taking template PDB-3f69 and modelled protein energy were minimized. Validation of the tertiary structure of PknA protein was done by PROCHECK.

RESULTS AND DISCUSSION

Primary Sequence Analysis of PknA

The sequence ofPknA, as given below, contains 431 AA with molecular weight of 45597 Da.

>sp|P65726|PKNA_MYCTU Probable serine/threonine-protein kinase PknA OS=Mycobacterium tuberculosis GN=PknA **(Table 1)**.

FASTA Sequence of PknA

MSPRVGVTLSGRYRLQRLIATGGMGQV
WEAVDNRLGRRVAVKVLKSEFSSDPEFI
ERFRAEARTTAMLNHPGIASVHDYGESQ
MNGEGRTAYLVMELVNGEPLNSVLKRT
GRLSLRHALDMLEQTGRALQIAHAAGL
VHRDVKPGNILITPTGQVKITDFGIAKAV
DAAPVTQTGMVMGTAQYIAPEQALGHD
ASPASDVYSLGVVGYEAVSGKRPFAGD
GALTVAMKHIKEPPPPLPPDLPPNVRELI
EITLVKNPAMRYRSGGPFADAVAAVRA
GRRPPRPSQTPPPGRAAPAAIPSGTTARV
AANSAGRTAASRRSRPATGGHRPPRRTF
SSGQRALLWAAGVLGALAIIIAVLLVIKA
PGDNSPQQAPTPTVTTTGNPPASNTGGT
DASPRLNWTERGETRHSGLQSWVVPPTP
HSRASLARYEIAQ

Table 1: Physicochemical Properties of PknA Shown by ProtParam

No. of amino acids	**431**
Molecular weight	**45597Da**
Total number of negatively charged residues (Asp + Glu)	**32**
Total number of positively charged residues (Arg + Lys)	**50**
Total no. of atoms	**2470**
Theoretical pI	**10.63**
Estimated half-life	**30 hours**
Instability index	**44.53**
Aliphatic index	**81.35**
Grand average of hydropathicity (GRAVY)	**-0.270**

NOTE: Ext. coefficient 33920; Abs 0.1% (=1 g/l) 0.744

The extinction coefficient indicates how much light a protein absorbs at a certain wavelength. It is useful to have an estimation of this coefficient for following a protein which a spectrophotometer when purifying it. From the molar extinction coefficient of tyrosine, tryptophan and cystine (cysteine does not absorb appreciably at wavelengths >260 nm, while cystine does) at a given wavelength, the extinction coefficient of the

native protein in water can be computed. The half-life is a prediction of the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell. The instability index provides an estimate of the stability of your protein in a test tube. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable. The instability index of our protein is 44.53.So the protein is unstable. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermostability of globular proteins. The GRAVY value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.

Secondary Structure Prediction

The program gives two outputs, one giving the sequence and the predicted secondary structure in rows, H=helix, E=extended or beta strand and C=coil; the second gives the probability values for each secondary structure at each amino acid position.

Prediction Result of GOR4 (Figure 1 & 2)

```
        10         20         30         40         50         60         70
         |          |          |          |          |          |          |
    MSPRVGVTLSGRYRLQRLIATGGMGQVWEAVDNRLGRRVAVKVLKSEFSSDPEFIERFRAEARTTAMLNH
    cccceeeeeccchhhheeeecccccchhhhhhhcccccceeeeeecccccchhhhhhhhhhhhhhhhhcc
    PGIASVHDYGESQMNGEGRTAYLVMELVNGEPLNSVLKRTGRLSLRHALDMLEQTGRALQIAHAAGLVHR
    ccceeecccccccccccchhhhhhhhccccccchhhhhccchhhhhhhhhhhhhhhhhhhhhhhhhhhhhc
    DVKPGNILITPTGQVKITDFGIAKAVDAAPVTQTGMVMGTAQYIAPEQALGHDASPASDVYSLGVVGYEA
    cccccceeeecccceeeehhhhhhhhccccecccceecchhhcccchhhhccccccccccceeeeceeeeec
    VSGKRPFAGDGALTVAMKHIKEPPPPLPPDLPPNVRELIEITLVKNPAMRYRSGGPFADAVAAVRAGRRP
    ccccccccccchhhhhhhhccccccccccccccchhhhhhhhccccccecccccchhhhhhhhccccc
    PRPSQTPPPGRAAPAAIPSGTTARVAANSAGRTAASRRSRPATGGHRPPRRTFSSGQRALLWAAGVLGAL
    ccccccccccccccccccccchhhhhhhhcccchhhhhccccccccccccccccccchhhhhhhhhhhhhh
    AIIIAVLLVIKAPGDNSPQQAPTPTVTTTGNPPASNTGGTDASPRLNWTERGETRHSGLQSWVVPPTPHS
    hhhhhhhhhhccccccccccccccceeeecccccccccccccccccccccccccccceeecccccceecccccc
                                RASLARYEIAQ
                                hhhhhheeeec
                          Sequence length:   431
                                GOR4:
    Alpha helix      (Hh) :   151 is  35.03%
    3₁₀ helix        (Gg) :     0 is   0.00%
    Pi helix         (Ii) :     0 is   0.00%
    Beta bridge      (Bb) :     0 is   0.00%
    Extended strand  (Ee) :    54 is  12.53%
    Beta turn        (Tt) :     0 is   0.00%
    Bend region      (Ss) :     0 is   0.00%
    Random coil      (Cc) :   226 is  52.44%
    Ambigous states  (?)  :     0 is   0.00%
    Other states          :     0 is   0.00%
```

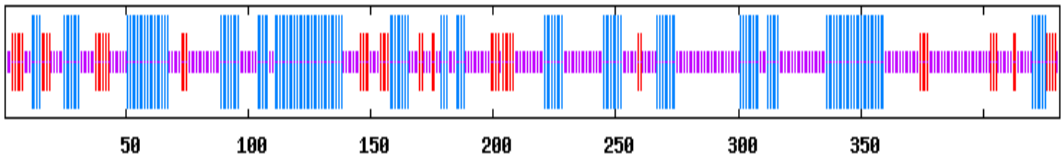

Figure 1: Prediction Result of GOR4

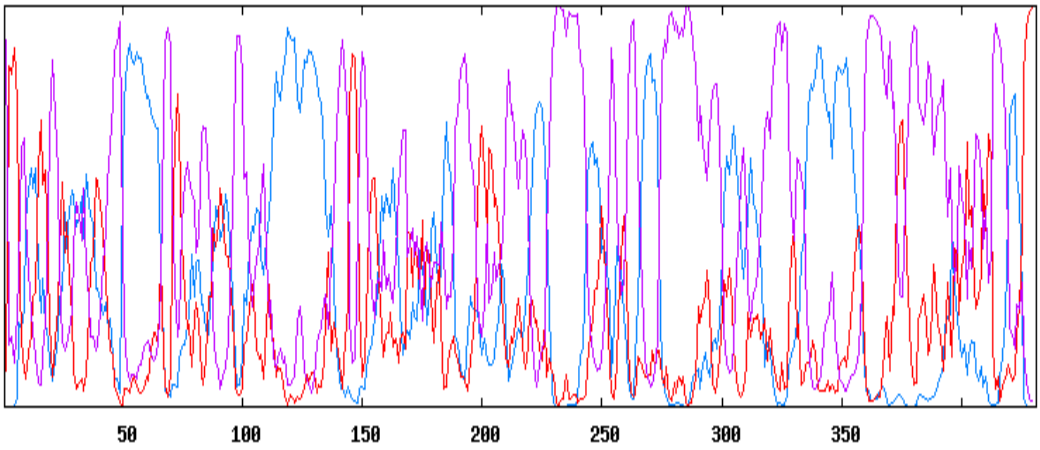

Figure 2: Prediction Result of GOR4

Prediction Result of SOPMA (Figure 3 & 4)

```
        10         20         30         40         50         60         70
         |         |          |         |          |          |          |
MSPRVGVTLSGRYRLQRLIATGGMGQVWEAVDNRLGRRVAVKVLKSEFSSDPEFIERFRAEARTTAMLNH
ccccccehtcceeeeeeecttcchhhhhhhhhhhhhhhhhhhhhhhcccchhhhhhhhhhhhhhhhccc
PGIASVHDYGESQMNGEGRTAYLVMELVNGEPLNSVLKRTGRLSLRHALDMLEQTGRALQIAHAAGLVHR
tteeeeeecccccccccccheeeeehcttcchhhhhhttccccchhhhhhhhhhhhhhhhhhttteeec
DVKPGNILITPTGQVKITDFGIAKAVDAAPVTQTGMVMGTAQYIAPEQALGHDASPASDVYSLGVVGYEA
cccttceeeettcceeeeehcccchhhhhccccctteeecccccccccccccccccccceeehhhhhhhh
VSGKRPFAGDGALTVAMKHIKEPPPPLPPDLPPNVRELIEITLVKNPAMRYRSGGPFADAVAAVRAGRRP
httcccccccchhhhhhhcccccccccccchhhhhhhhhhhhttttcccccccchhhhhhhhhttccc
PRPSQTPPPGRAAPAAIPSGTTARVAANSAGRTAASRRSRPATGGHRPPRRTFSSGQRALLWAAGVLGAL
ccccccccccccccccccccccceecccccccccccccccccccccccccccttthhhhhhhhhhhhhh
AIIIAVLLVIKAPGDNSPQQAPTPTVTTTGNPPASNTGGTDASPRLNWTERGETRHSGLQSWVVPPTPHS
hhhhhheeeecccccccccccccceeecccccccccccccccceeecccccccttccceeeecccccc
                    RASLARYEIAQ
                    chhhhhhhhhh
                Sequence length:   431
                      SOPMA:
 Alpha helix      (Hh):   140 is  32.48%
 3₁₀ helix        (Gg) :    0 is   0.00%
 Pi helix         (Ii) :    0 is   0.00%
 Beta bridge      (Bb) :    0 is   0.00%
 Extended strand  (Ee) :   54 is  12.53%
 Beta turn        (Tt) :   29 is   6.73%
 Bend region      (Ss) :    0 is   0.00%
 Random coil      (Cc) :  208 is  48.26%
 Ambigous states  (?)  :    0 is   0.00%
 Other states          :    0 is   0.00%
```

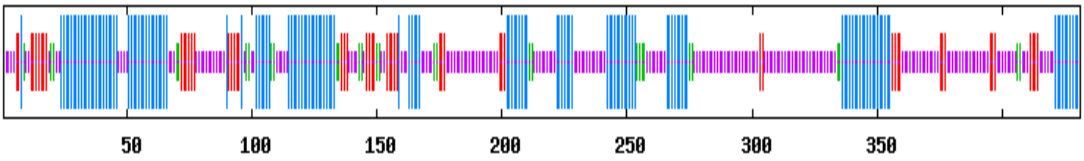

Figure 3: Prediction Result of SOPMA

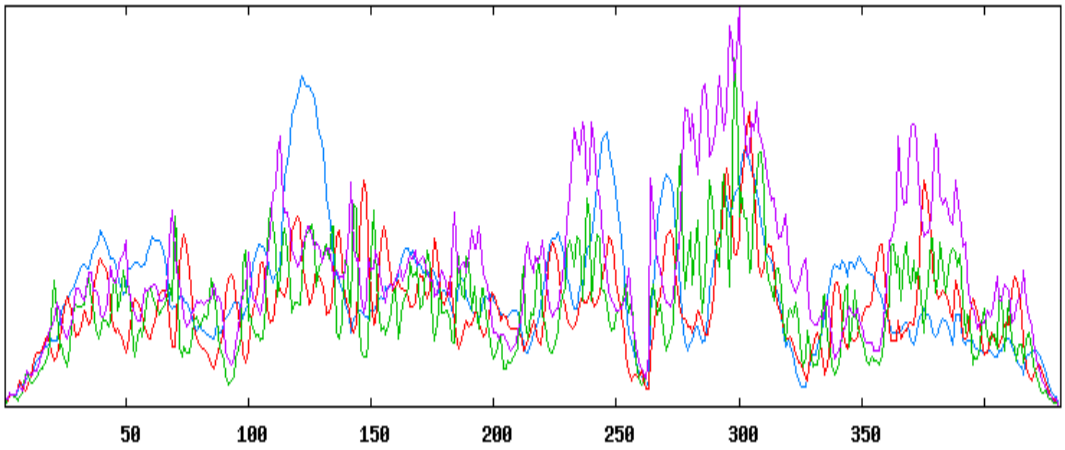

Figure 4: Prediction Result of SOPMA

```
Parameters: Window width: 17; Similarity threshold:8;Number of states :4
```

The results revealed that random coil has the highest percentage and beta strand has the lowest percentage.

Tertiary structure prediction

In protein structure prediction, homology modeling, also known as comparative modeling, is a class of methods for constructing an atomic-resolution model of a query or target protein from its amino acid sequence. In this study approximately all homology modeling techniques rely on the identification of one or more known protein (template) structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. The sequence alignment and template structure are then used to produce a structural model of the target. Since protein structures are more conserved than protein sequences, detectable levels of Sequence similarity usually involve significant structural similarity.

Modeled Structure of PknA (Figure 5)

Figure 5: Modeled Structure of PknA

Ramachandran Plot showing Model validation (Figure 6)

Three dimensional structure of PknA was successfully modeled using SWISS- MODEL. The predicted structure was further validated using prochek and checked for its reliability based on the Ramachandran plot, by fulfilling the required rules. Structural analysis was performed and figures representations were generated with Swiss PDB Viewer **[24]**. In the modeled structure 87.9% residues are in most favored regions, 10.3%in additional regions and 1.8% in generously allowed regions. But no residues are in the disallowed regions.

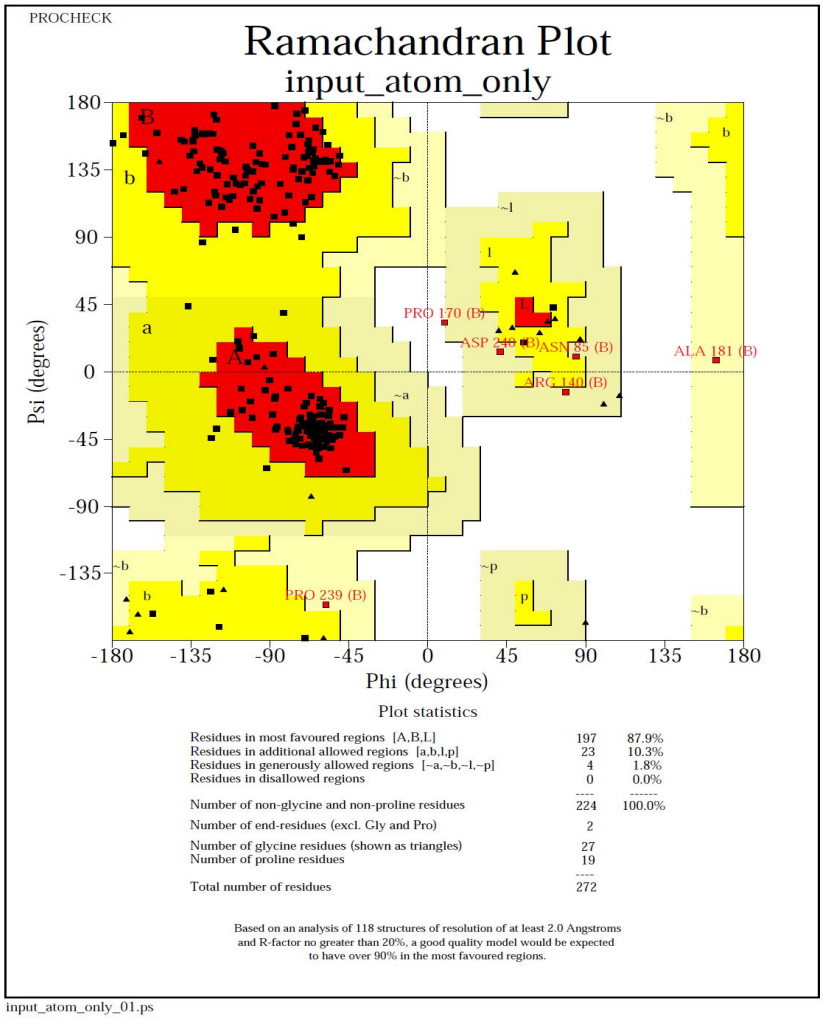

Figure 6: Ramachandran Plot showing Model validation

CONCLUSION

Protein phosphorylation is a key mechanism by which environmental signals are transmitted to control protein activities in both eukaryotic and prokaryotic cells. Prokaryotic STPKs regulate various cellular functions, such as developmental processes, primary and secondary metabolism, stress responses and biofilm formation. *In-silico* analysis of the genome sequence of *Corynebacterium glutamicum* predicted the presence of four different STPKs. STPKs also play a role in the virulence of many bacterial pathogens such as *streptococci, Staphylococcus aureus, Mycobacterium tuberculosis, Yersinia spp.*, and *P. aeruginosa*., Bacterial pathogens containing eSTKs have developed a diversity of strategies to manipulate host signalling pathways that aim to either weaken an effective immune response, or to create a suitable environment in which the invading

pathogen(s) can survive, propagate and flourish. The modelled structure of pknA will be helpful for the structural interaction study .the interaction will help us to understand the interface residues between the host and the pathogen, protein which will be helpful for the drug development against *M. tuberculosis.*

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