

1 **Understanding properties of the master effector of phage shock operon in**
2 ***Mycobacterium tuberculosis* via bioinformatics approach**

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7 **Keywords:** *Mycobacterium tuberculosis*, Phage shock protein, Structure modeling,
8 Dynamics simulation.

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17 **NOTE:** An expanded version of this manuscript is under-preparation. The current pre-print
18 version (v.01) of this manuscript may contain grammatical & proofreading mistakes. Errors
19 and omissions excepted.

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1 **Abstract**

2 The phage-shock-protein (psp) is a part of the psp operon, which assists in safeguarding the
3 survival of bacterium in stress and shields the cell against proton motif force challenge. It is
4 strongly induced by bacterium allied phages, improperly localized mutant porins and various
5 other stresses. Master effector of the operon, PspA has been modeled and simulated,
6 illustrating how it undergoes significant conformational transition at the far end in
7 *Mycobacterium tuberculosis*. Association of this key protein of the operon influences action
8 of Psp system on the whole. We are further working on the impact of phosphorylation
9 perturbation and the impact of structural fluctuations during complex formation of PspA with
10 other moieties of interest.

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1 We hereby report *ab initio* structure model of the *Mycobacterium tuberculosis* phage shock
2 protein A (PspA). PspA is the central constituent of bacterial stress response machinery,
3 encoded by phage shock operon (Huvet et al., 2010). PspA, regulates not only it's own
4 transcription but that of the whole operon as well (Elderkin et al., 2005; Male et al., 2014).
5 PspA is inferred to be a dual-function protein (Guegwen et al., 2009; Jovanovic et al., 2014)
6 and localized amid cytoplasmic and inner membrane interface of the bacterium (Engl et al.,
7 2009). It is responsible for maintaining the cell membrane integrity along with restoration of
8 the proton motive force (Male et al., 2014; Engl et al., 2009; Wan et al., 2015).

9 *Mycobacterium tuberculosis* is a pathogenic bacterium responsible for causing disease in
10 humans and veterinary species (Sakamoto, 2012). A lot of work has been carried out on
11 *Mycobacterium* phages for therapeutic purpose. However, to the best of our knowledge, no
12 report of the phage shock protein analysis for this pathogen exists at the moment. We
13 retrieved PspA protein sequence of *Mycobacterium tuberculosis* from the Uniprot database
14 with Accession number: R4M912 and analyzed the sequence and the structure using
15 computational tools. Although, PspA belongs to the highly conserved PspA/IM30 family but
16 *Mycobacterium tuberculosis* PspA shared a very low sequence homology with *Escherichia*
17 *coli* PspA, revealed using Clustal Omega (Fig. 1) with seeded guide trees and HMM profile-
18 profile technique for alignment generation at the backend (Sievers et al., 2011). The
19 secondary structure analyzed by PROMOTIF tool (Hutchison and Thornton, 1996) revealed
20 that the protein consisted of 9 helices, 6 helix-helix interacts, 14 β -turns and 3 α -turns (Fig.
21 2A).

22 Due to low homology with experimentally determined structures available in the RCSB
23 Protein databank, 3D structure (Fig. 2B) was modeled by I-TASSER (Roy et al., 2010; yang
24 et al., 2015) using *Escherichia coli* PspA as a template (PDB ID: 4WHE). The C-score based
25 on the significance of template alignment threading and simulations of the structure assembly

1 convergence parameters of the model chosen for analysis was -1.29 (lies between ideal range
2 of -5 to 2), indicating good quality model. Estimated root mean square deviation of the
3 predicted model from the *Escherichia coli* model was $7.8 \pm 4.4 \text{ \AA}$. The constructed structure
4 resembled a helical bundle with important binding site residues predicted to occur at position
5 66, 69, 70, 73, 92, 96, 99, 100, 102 and 103. Despite low sequence conservation, the structure
6 is however, well conserved due to the underlying fact that the protein folds remain conserved
7 in similar function proteins. PspA structure in *Escherichia coli* is known to self-assemble into
8 ring (Standar et al., 2008) or striated and indented rod-shaped complexes (Male et al., 2014)
9 based on electron microscopy and helical rods based on X-ray crystallography analysis
10 (Osadnik et al., 2015). PspA homologue LiaH in *Bacillus subtilis* (Wolf et al., 2008) and
11 holins of bacteriophage lambda (Savva et al., 2010) have also been reported to self-assemble
12 to rod-like structures from ring shaped protein complexes. The *Mycobacterium tuberculosis*
13 PspA is also rod shaped and it is implied that these rod-like structures could form a support
14 framework and aid in the maintenance of membrane integrity during phage shock response
15 (Male et al., 2014).

16 CABS-flex procedure based on the well-established coarse-grained CABS protein model
17 (Fraga et al., 2014) was employed for the fast simulation of near-native dynamics of PspA.
18 CABS is a computationally efficient alternative to classical all-atom molecular dynamics
19 (Jamroz et al., 2013). The 3D modeled structure was input and used as a starting point for the
20 all-atom, explicit water, 10-nanosecond dynamic simulation. Analysis was carried out at the
21 backend and automatically analyzed trajectory (Fig. 3) was obtained to study the dynamic
22 behaviour of protein. A set of eight (all-atom) protein model sets were obtained with global
23 distance test score ranging from 0.6-0.7. Most dominant structural fluctuations appeared at
24 the last beta turn region including 8th and 9th helix. Relative propensity of protein residues to
25 deviate from an average dynamics structure increased substantially at the ending helices with

1 fluctuation increasing from 100 Å at residue 160 to to >600 Å at residue 172. Understanding
2 of flexibility of PspA can be of aid in research areas as molecular evolution (Boehr et al.,
3 2009).

4 Phage infection has also been demonstrated to induce substantial fluctuations in host protein
5 phosphorylation (Rieul et al., 1987; Russel and Model, 2006) and this was suggestive of
6 PspA potential for phosphorylation as well. NetPhos Bac 1.0 (Miller et al., 2009) was used
7 for prediction of possible phosphorylation residues. Seven serine residues (S144, S149, S156,
8 S157, S158, S164, S166) were predicted to have phosphorylation potential based on neural
9 network approach. However, none of these exhibited a knack to occur on predicted binding
10 residues and hence, their exact role in PspA function of *Mycobacterium tuberculosis* yet
11 remains to be elucidated.

12 Our findings pave way for further experimental studies and are of aid in understanding the
13 *Mycobacterium tuberculosis* PspA response to the extracytoplasmic stresses that may damage
14 the cytoplasmic membrane. We have used computational approach for the prediction of 3D
15 structure of this protein but to furthur understand the function of the rod-like structure of
16 *Mycobacterium tuberculosis* PspA, additional studies are required which can confirm and
17 enhance the reported information along with elucidation of in depth biological function and
18 interactions of *Mycobacterium tuberculosis* PspA.

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1 **Figure Legends**

2 Figure 1. Multiple sequence alignment of the *Escherichia coli* and *Mycobacterium*
3 *tuberculosis* PspA. Conserved residues are shown in red color. Helices are denoted by
4 squiggles at the top of the alignment. Solvent accessibility is depicted by a bar below the
5 sequence (blue = accessible, cyan = intermediate, white = buried).

6 Figure 2. (A) Secondary structure of *Mycobacterium tuberculosis* PspA (helices labelled H1,
7 H2...H9). β depicts beta turn and γ is for gamma turn. (B) 3D structure of *Mycobacterium*
8 *tuberculosis* PspA.

9 Figure 3. (A) Structural flexibility profile of simulated *Mycobacterium tuberculosis* PspA
10 with fluctuations for individual protein residues shown via red line. The output is based on
11 all-atom model via trajectory clustering. (B) Refinement of the model and superpositioning
12 (Provided 3D model as base) is centred on maximum likelihood superposition method of
13 THESEUS (Theobald and Wuttke, 2006).

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17 **Competing interests**

18 The authors declare that no competing interests exist.

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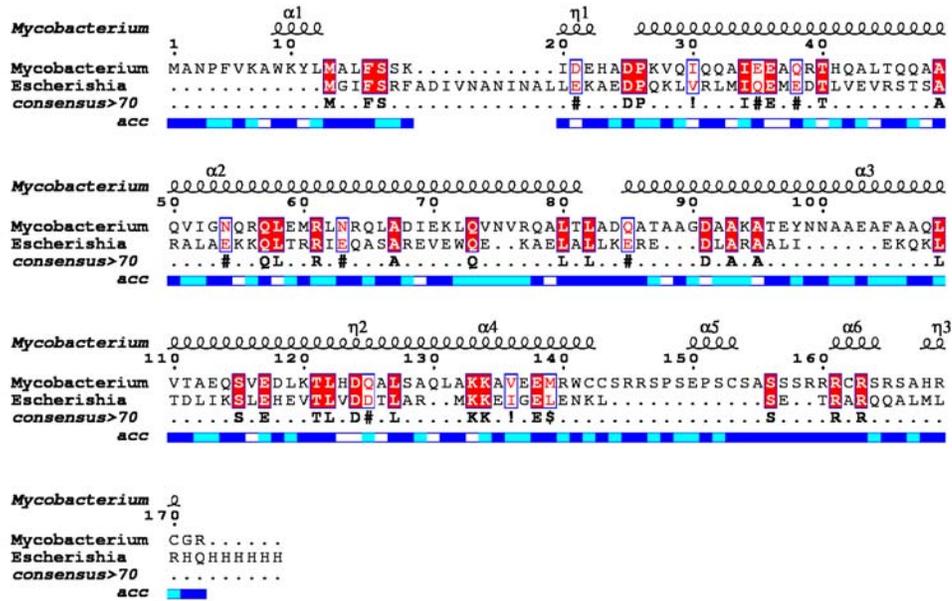
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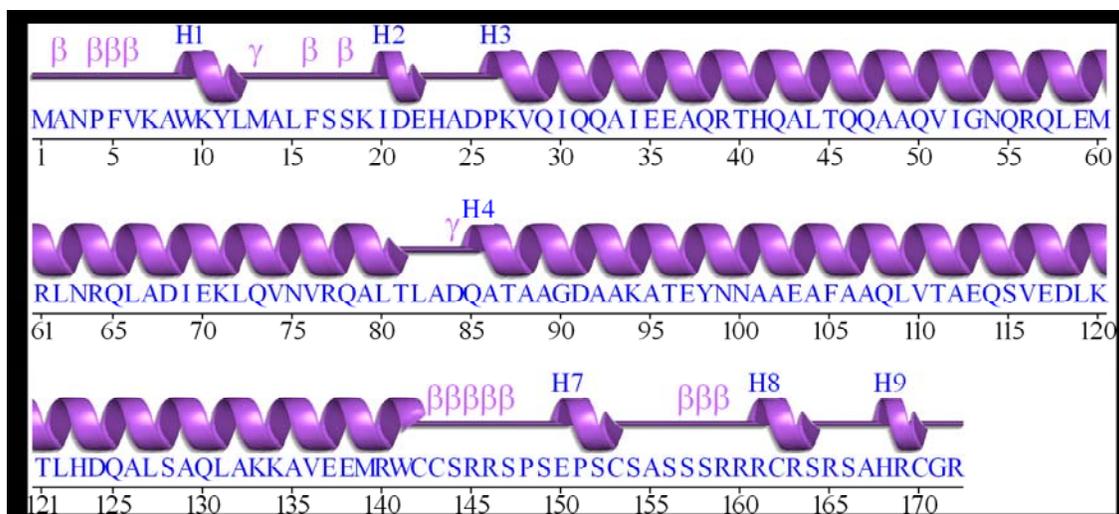
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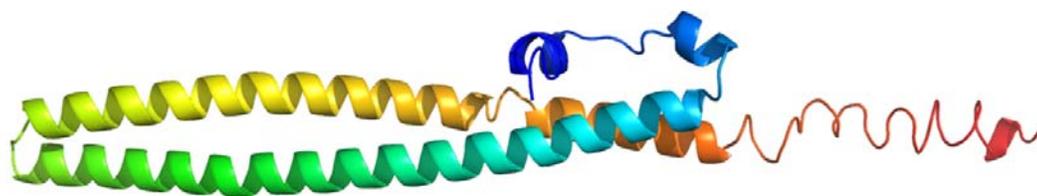
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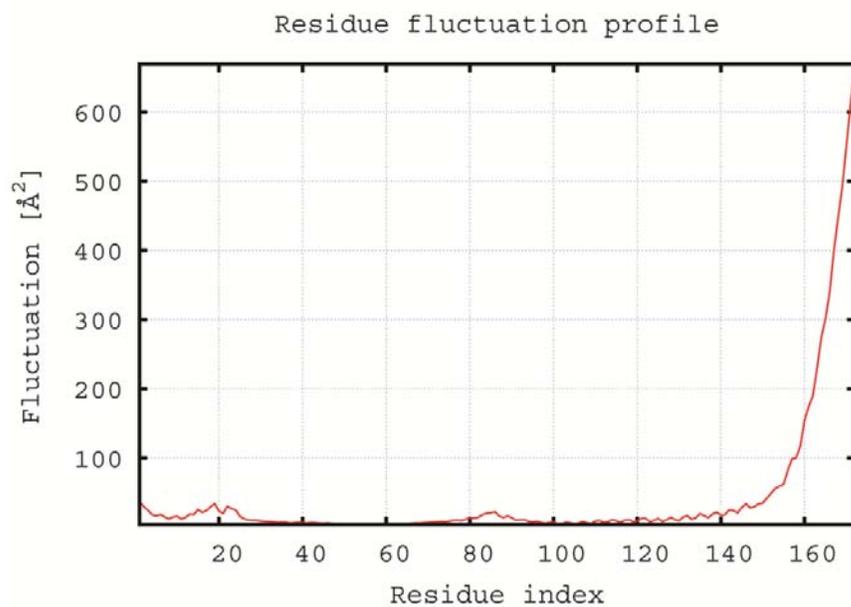
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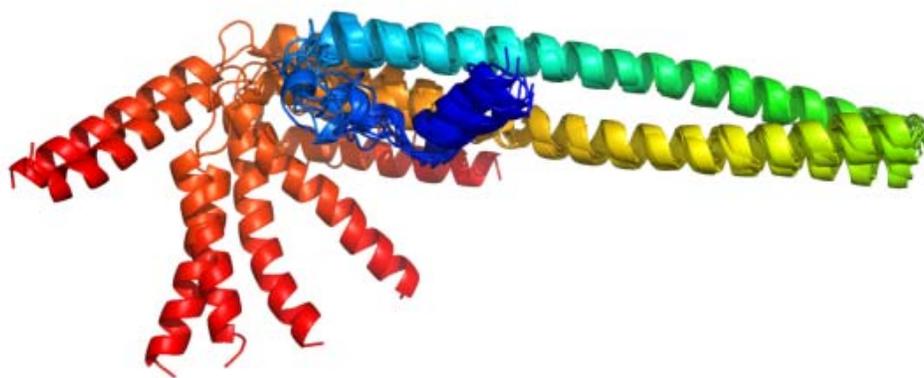
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